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INTRODUCTION TO THE SPECIAL ISSUE ON PHENOLOGICAL PATTERNS IN THE FLORA OF WESTERN NORTH AMERICA

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If there were ever a time to study plant phenology, the time is now. Change is upon us in every regard and as a Society focused on the natural history of the flora of western North America, this special issue is focused on understanding the phenological responses of plants in our region. Western North America contains a diverse and highly endemic flora (Burge et al. 2016) that is also highly endangered. There are many threats to the flora, including rapid land use changes and anthropogenic climate change, which pose a heightened threat to California's endemic flora and beyond. Rising temperatures, predicted by climate models, are being observed in the West (Parmesan and Yohe 2003; Kelly and Gouliden 2008), and precipitation patterns are becoming more variable (Swain et al. 2018). These changes are already impacting plants in this region, as shown in many of the articles presented here. Understanding how plant species, populations, and communities change over time and across space is critical to directing conservation efforts, land management, and future scientific inquiry.

Plant phenology—or the timing of life history events, such as bud break or flowering—is a critical component of an organism's biology, and links it to a myriad of other interacting organisms (such as Olliff-Yang and Mesler 2018; Mulder et al. 2021 *this issue*). What triggers plant phenological responses is a fundamental question of organismal biology and ecology, yet for most species, this remains a mystery. Recent advances in available data have made studying phenology of plants much more feasible, and we are pleased to have brought together a group of articles that advance our knowledge of the phenological patterns of our flora (Fig. 1).

At its core, phenological research asks a basic question about an organism: “What environmental factors (e.g., temperature or precipitation) induce changes in the timing of a life history event (e.g., leaf drop, bud burst, flowering, fruiting, or senescence)?” The availability of climate data for the entire United States via Climate NA and PRISM provides many environmental variables with which to look for correlations with the phenological trait of interest (PRISM Climate Group 2004; Wang et al. 2016).

Phenological data has traditionally come from *in situ* observations of plants in the field. However, to understand plant responses to *in situ* environmental conditions, observations must span many years, making these datasets rare. In this issue you will see the results of a 5-yr study of *Dirca occidentalis* (Graves and Gimondo 2021 *this issue*), a 10-yr study of *Calochortus plummerae* (Williams et al. 2021 *this issue*), a 30-yr dataset for six desert taxa (Zachmann et al. 2021 *this issue*), and a 30-yr study in *Quercus* (Koenig et al. 2021 *this issue*). Common gardens are also incredibly valuable for understanding how plants from different populations vary with respect to phenological responses. Two papers featured here present results from 30-yr old common gardens in *Quercus* (Papper and Ackerly 2021 *this issue*; Wright et al. 2021 *this issue*). These long-term projects require sustained effort over long time periods, and we are excited to present their findings in this issue.

A more recent way to generate phenological data is to engage the public in the process of making *in situ* observations of plants. The National Phenology Network empowers people to make phenological observations of species in the field and, as a network, volunteers currently monitor over 1,000 plant species with data going back to the early 2000's (USA National Phenology Network 2021). These are recorded and aggregated for research use. Armstrong-Herniman and Greenwood (2021 *this issue*) use these data to understand the flowering phenology of five oak species. Other networks, like the Winterberry Network in Alaska, provide data on fruit availability during the harsh winters of northern regions (Mulder et al. 2021 *this issue*). These crowd-sourcing approaches provide robust datasets to answer questions on larger spatial scales.

Herbarium specimens preserve phenological data of plants across time and space. Although most herbarium specimens were not generally collected with the purpose of conducting phenological research, the phenological status of a specimen can be seen by visually inspecting the specimen. The use of herbarium specimens to track the relationship between local climatic conditions and the collection dates of flowering specimens has a relatively short

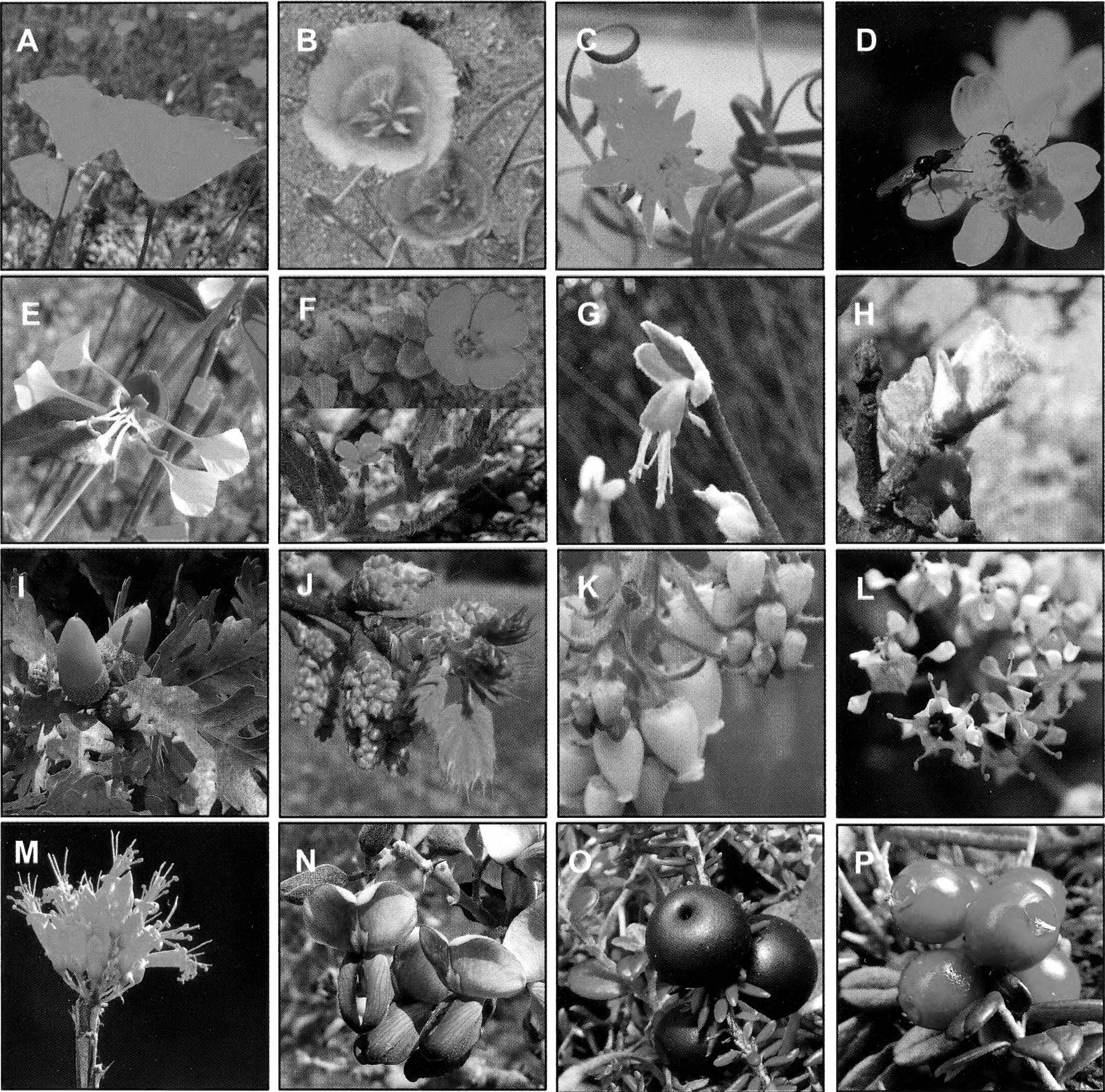


FIG. 1. Examples of plants included in this Special Issue on phenology, in order of appearance. (A) *Eschscholzia californica*, photo: Katelin Pearson; (B) *Calochortus plummerae*, photo: Kimberlyn Williams; (C) *Lasthenia gracilis*, photo: Emily Cox; (D) *Lasthenia californica*, photo: Alexander C. Yang; (E) *Clarkia unguiculata*, photo: Susan Mazer; (F) Bodega congeners *Camissoniopsis cheiranthifolia* (top) and *C. micrantha* (bottom), photos: Sharon Strauss and Keir Morse (respectively); (G) *Dirca occidentalis*, photo: William R. Graves; (H) *Quercus douglasii*, photo: Prahlad Papper; (I) *Quercus lobata*, photo: Walt Koenig; (J) *Quercus kelloggii*, photo: Wendy Armstrong-Herniman; (K) *Arctostaphylos andersonii*, photo: Tom Parker; (L) *Ceanothus cuneatus*, photo: Tom Parker; (M) *Fouquieria splendens*, photo: Rhonda Spencer; (N) *Olneya tesota*, photo: Mark Dimmitt; (O) *Empetrum nigrum*, photo: Anne Ruggles; (P) *Vaccinium vitis-idaea*, photo: Anne Ruggles.

history (Willis et al. 2017). Nevertheless, herbarium-based phenological studies are dramatically increasing the temporal scale of phenological research. The consortium of California Herbaria has a robust record of curating the state’s herbarium specimens and currently specimens are being imaged as part of the California Phenology Thematic Collections Network (Yost et al. 2019; <https://www.capturingcaliforniasflowers.org/>; NSF Project #1802163). Phenological status is being recorded on

each of these images. As of 2021, there are now over 1.3 million phenological records in the CCH2.org data portal, which are available for research use. These records will dramatically increase phenological research by creating a historical record going back to the 1800’s. Here, we present two papers that use phenological data from herbarium specimens. Mazer et al. (2021 *this issue*) used herbarium records spanning the last 115 yr for *Clarkia* species. Pearson et al. (2021 *this issue*) uses the historical record to

study how California's state flower, *Eschscholzia californica*, is responding across its range.

The variation observed in these papers and others clearly indicates that generalizations about shifts in phenology due to climatic changes are too early at this point. In this issue, Zachmann et al. (2021 *this issue*) find that for some desert taxa, warming promotes earlier flowering, yet individual species still varied in their response to several climate variables. Williams et al. (2021 *this issue*) find that for a rare geophyte, rainfall and time since fire, were most correlated with flowering. Armstrong-Herniman and Greenwood (2021 *this issue*) find that winter rainfall drives budburst in five long lived perennial tree species (*Quercus* spp.). Yet for one of the same species, *Quercus lobata*, warming temperatures were shown to advance flowering times (Koenig et al. 2021 *this issue*). For two other woody perennials, *Ceanothus* and *Arctostaphylos*, there was no evidence for an advance in flowering time over that last century (Parker 2021 *this issue*). For the winter flowering species *Dirca occidentalis*, fall rainfall and fall and winter temperatures drove flowering (Graves and Gimondo 2021 *this issue*). Water availability drove the intensity and duration of flowering of *Lasthenia gracilis*, a spring annual (Cox and Olliff-Yang 2021 *this issue*). In a second paper on *Lasthenia*, we find that germination date and competition from neighboring plants are important predictors of the flowering season of *L. californica* (Olliff-Yang and Ackerly 2021 *this issue*). In *Eschscholzia californica*, long term temperatures were more important than long term precipitation in predicting flowering time (Pearson et al. 2021 *this issue*). Plants may also exhibit a plastic response to flowering, whereby changes in the environment can be tolerated to a certain extent. We show here that responses are variable among close relatives, as Mazer et al. (2021 *this issue*) found in *Clarkia* and Strauss et al. (2021 *this issue*) found across a wide variety of taxa in a community context in Bodega Bay, California. We also see variation in phenological responses within populations of the same species, as in the Blue Oak (Papper and Ackerly 2021 *this issue*), and California Poppy (Pearson et al. 2021 *this issue*). Given this variation, future predictions across ecosystems are difficult to make at this time. More widely available data for a larger number of taxa and across more temporal and geographic scales will help to illuminate general trends, if there are any.

As more phenological studies are conducted, we can begin to make comparisons across taxonomic groups and across climates. Many papers in this issue are focused on plants in the Mediterranean climate of western California. These responses can be contrasted with responses from the far north (Alaska – Mulder et al. 2021 *this issue*) to those of the southwest (Sonoran Desert – Zachmann et al. 2021 *this issue*).

There has been extensive work done on phenology across the world, but we are still just beginning to

understand the complexity of timing that can occur within and between species, across landscapes and in different ecological contexts. This understanding is essential as climate change continues to alter the environmental cues that plants are responding to, and many species may need to shift rapidly to track optimal conditions.

Regardless of the drivers of a phenological event, the timing of plant resources—such as nectar, pollen, and fruit—strongly influence interacting organisms. For example, in this issue we see that when the fall season is extended and fruit ripen earlier, food resources in the winter and spring will be lower (Mulder et al. 2021 *this issue*). Environmental variables influence flowering season duration (Cox and Olliff-Yang 2021 *this issue*; Olliff-Yang and Ackerly 2021 *this issue*) and determine the conditions when flowers may be available for pollination (Graves and Gimondo 2021 *this issue*), which can result in higher or lower overlap in time with active pollinator species. Additionally, differences in timing between species and across habitats will have consequences for pollination and competition (Mazer et al. 2021 *this issue*; Strauss et al. 2021 *this issue*). For these reasons, shifts in phenology with climate change may have cascading effects within ecosystems, and will be important to continue to track.

In this issue, fourteen works are presented, covering a wide array of taxa representative of a small slice of our extremely diverse flora (Fig. 1). These manuscripts include historical, observational, and experimental studies of the timing of various plant stages, from leaf out and flowering to fruit set. Many authors from across western North America and beyond contributed manuscripts for this issue, and we are pleased to present a collection of papers spanning a diverse set of species.

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PHENOLOGICAL TRENDS IN THE CALIFORNIA POPPY (*ESCHSCHOLZIA CALIFORNICA*): DIGITIZED HERBARIUM SPECIMENS REVEAL INTRASPECIFIC VARIATION IN THE SENSITIVITY OF FLOWERING DATE TO CLIMATE CHANGE

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ABSTRACT

Herbarium specimens provide a critical source of phenological data that can be used to identify the direct and indirect drivers of variation in flowering date within and among species. Specimen-based phenological research in California has been accelerated by digitization efforts such as the California Phenology Network, which has scored and archived the phenological status of over 1.4 million specimens to date. Using this new data source in the Consortium of California Herbaria's CCH2 data portal, we obtained data from 993 specimens of the iconic California Poppy, *Eschscholzia californica* Cham., along with climate data representing all collection sites. Our goal was to determine how long-term and interannual climate variation affect flowering dates, and whether the magnitude of phenological sensitivity to climate varies across the species' range. We found that specimens collected from chronically warm or dry sites flowered relatively early, and flowering date was more sensitive to long-term mean temperature than to long-term mean precipitation. Independent of these effects of long-term conditions, flowering date in *E. californica* was sensitive to interannual variation in seasonal precipitation, but the direction of this effect depended on the season in which the precipitation occurred. Specimens sampled from sites experiencing warmer-than-average springs in the year of collection flowered 2.7–3.3 days earlier for every 1°C increase in spring temperature relative to long-term mean spring temperature. The magnitude of these effects, however, varied across the range of *E. californica*, with greater sensitivity to temperature in relatively cooler regions and no discernible sensitivity in relatively warm regions. Consistently, California Poppies exhibited significant phenological advancement over the last 120 years, but this advancement was restricted to the cooler portions of its range. Our results provide one of the first accounts of intraspecific variation in both phenological sensitivity to climate and the magnitude of phenological shifts over time, and demonstrate that, for a single species, location- or population-specific estimates of phenological sensitivity or of temporal trends in phenology might not accurately predict phenological responses to climate change in other locations throughout its range. In this study, we highlight the utility and promise of herbarium specimens for addressing novel questions about the phenological responses of plants to climate trends.

Key Words: California Poppy, climate change, flowering date, phenology, CCH2, Consortium of California Herbaria, temperature, precipitation

Shifts in plant phenological events (e.g., flowering or fruiting times) are well-documented effects of climate change across the globe (Parmesan and Yohe 2003; Fitchett et al. 2015; Scheffers et al. 2016; Piao et al. 2019). Because many organisms and ecological processes depend on the timing of plant phenological events, shifts in these events may affect species interactions and coexistence (Hegland et al. 2009; Kharouba et al. 2018; Rudolf 2019), ecosystem

functioning (Richardson et al. 2010), and plant fitness (Mohan et al. 2019), especially since the phenological sensitivities of individual plants or populations may differ from those of the species with which they interact, even indirectly (Thackeray et al. 2016). For example, when elderberries and sockeye salmon became phenologically synchronized in Alaska due to differing responses to warmer spring temperatures, Kodiak brown bears consumed more

elderberries and fewer salmon, altering the mortality rates of salmon and consumption of elderberry fruits (Deacy et al. 2017).

Differences among plant species in phenological sensitivity to climate have been widely reported (Wolkovich et al. 2012; Delgado et al. 2020; Parker 2021). However, the influence of climate on phenological events may also vary within species (Song et al. 2020), further complicating the prediction of the phenological effects of upcoming climate change, as these effects may be highly population- or region-specific. For several reasons, different parts of a widespread species' range might exhibit different degrees of phenological sensitivity to local, long-term climatic conditions or to inter-annual variation in climate. For example, with respect to temperature-sensitivity, in portions of a species' range where growth and reproduction are limited by cooler temperatures, we may expect populations to evolve strong sensitivity to inter-annual variation in temperature, resulting in earlier flowering in warmer-than-average years relative to "normal" years. Such phenotypic plasticity may be favored by natural selection because it enables individual plants to take advantage of the longer growing period that characterizes relatively warm years. By contrast, in portions of a species' range characterized by chronically warm, frost-free conditions, populations may evolve to flower in response to cues other than temperature, simply because local temperature might not be correlated with any particular risk through which natural selection would operate. Alternatively, if the relationship between local temperature or rainfall and flowering date is non-linear within a given species, then the phenological sensitivity to variation in these parameters would also necessarily change across temperature and/or precipitation gradients. In this case, even in the absence of genetically-based variation in phenological sensitivity to climate across the species' range, we would observe regional variation in quantitative estimates of such sensitivity. In addition, if biotic factors that may influence the evolution of flowering time, such as the timing of pollinator availability or the timing of floral predation, covary with climate in some portions of the range but not others, then phenological sensitivity to climate would also appear to vary geographically. Whatever process drives spatial variation in phenological sensitivity to climate, it is important to document it, as accurate, region-specific forecasts of climate-driven phenological change depend upon both accurate predictions of the magnitude and direction of upcoming climate change and accurate estimates of phenological sensitivity to changes in temperature and precipitation.

To date, intraspecific variation in phenological sensitivity has been assessed in only a few taxa (Wang et al. 2015; Matthews and Mazer 2016; Prev  y et al. 2017; Park et al. 2018; Song et al. 2020), and the processes generating such variation are poorly understood. In the current study, we leverage the

broad spatiotemporal coverage of digitized herbarium specimens of California Poppy (*Eschscholzia californica* Cham.) to detect and to interpret geographic variation in phenological sensitivity to three sources of climatic variation: geographic variation in long-term mean climatic conditions, inter-annual variation in temperature and rainfall, and seasonal variation in temperature and rainfall.

Our understanding of phenological shifts in plants has been derived largely from recurrent, location-specific observational records of living plants (Wolkovich et al. 2012, and references therein). These studies have advanced our understanding of the phenological responses of many species in a relatively small number of locations, but extrapolating to other regions or taxa is complicated by the magnitude of variation observed among taxa (Calinger et al. 2013; Park et al. 2018; but see Mazer et al. 2021). Furthermore, because observational phenological studies have predominantly estimated species-specific phenological sensitivities to climate using records from few locations, it is typically unclear whether they accurately represent the phenological sensitivity of a species in other locations within its range. To further understand the factors influencing phenological shifts within and among taxa, large quantities of data are required for many taxa representing different functional groups, with extensive coverage across time and space. Only in recent years have such data become available through herbarium digitization efforts (e.g., Yost et al. 2018) and observational data-collecting networks (e.g., the USA National Phenology Network, usanpn.org), enabling investigators to evaluate the causes and consequences of phenological shifts in rare or understudied plant communities (e.g., Munson and Sher 2015), across a more diverse set of taxa (e.g., Park and Mazer 2018), and across populations within individual species (e.g., Song et al. 2020).

Herbarium specimens, in particular, have emerged as a key source of phenological data, empowering studies across large spatiotemporal scales and for diverse and understudied plant, lichen, algal, and fungal taxa (Davis et al. 2015; Willis et al. 2017; Jones and Daehler 2018). Herbarium specimens have been collected across the world for several hundred years as vouchers for studies in taxonomy, biogeography, ecology, and other fields; their immense value for phenological research is a serendipitous by-product of this foundational work. Herbarium specimens provide a "snapshot" of the phenological status of a given plant in a specific location on a certain date. In aggregate, analyses of specimen-based data have revealed how plants in different regions and environments (Park et al. 2018, Song et al. 2020), taxonomic groups (Calinger et al. 2013; Park et al. 2018), and functional groups (Calinger et al. 2013; Bertin 2015) respond phenologically to climate. In some cases, this work has identified the climate variables and seasons that are most influential for the studied species (Matthews and Mazer

2016; Park and Mazer 2018). Phenological estimates derived from herbarium specimens have demonstrated high reliability and congruence with those from other sources (Miller-Rushing et al. 2006; Panchen et al. 2012; Davis et al. 2015) despite known sampling biases (Daru et al. 2017).

Specimen-based phenological research, and specimen-based research in nearly all fields, has been greatly accelerated by digitization: imaging, transcribing label data, and georeferencing the collection locations of herbarium specimens (Willis et al. 2017). The National Science Foundation (NSF) has funded the mass digitization of natural history collections in the United States through the Advancing Digitization of Biodiversity Collections (ADBC) program, established in 2010. Over the last ten years, more than 125 million specimen records have been made freely available electronically (e.g., via iDigBio, <https://www.idigbio.org/>). These activities not only allow greater access to basic specimen data, but they also allow researchers to harvest phenological data on a larger scale and explore new avenues of research (Soltis 2017) such as those involving machine learning (Pearson et al. 2020).

Herbarium specimen digitization in California has a relatively long history, owing in part to the Consortium of California Herbaria (CCH), founded in 2003. Digitization activities have been recently accelerated by the California Phenology Thematic Collections Network (CAP TCN; <https://www.capturingcaliforniasflowers.org/>), funded by the NSF in 2018. This four-year project funds the digitization of nearly one million California vascular plant specimens (Yost et al. 2019). As a result, the project's new data portal, CCH2 (<https://cch2.org/>) currently contains phenological data for over 1.4 million publicly accessible specimen records. By making these data available, the CAP TCN aims to facilitate phenological research that will improve our understanding of future changes to the California flora and help determine how potential threats to California biodiversity, ecology, and agriculture may be mitigated.

In this study, we used specimen occurrence data and newly created phenological data for 993 specimens of the California Poppy (*Eschscholzia californica*), the state flower of California, available in CCH2 to examine the effects of climate on the timing of flowering across the range of this iconic species. Using these data, we (1) identified the seasonal windows during which temperature and precipitation best predict flowering date of *E. californica*, (2) determined the magnitude and direction of the effects on flowering date of long-term temperature and precipitation, (3) assessed the effects on flowering date of interannual variation in seasonal temperature and precipitation, (4) detected whether temperature and precipitation have changed over time throughout the range of *E. californica*, and (5) examined whether the phenological sensitivity of this species to interannual climate variation or rates of phenological

change over time vary among regions within its range. Additionally, although most studies have reported stronger phenological effects of interannual variation in temperature than of precipitation (Abu-Asab et al. 2001; Gordo and Sanz 2010; Hart et al. 2014; but see Matthews and Mazer 2016; Munson and Long 2017; Wolf et al. 2017), it is unclear whether the relative influence of these climatic factors is the same for species in water-limited regions, such as the native range of *E. californica*, or whether their relative importance is stable across a species' range. Therefore, we also compared the relative effects of interannual variation in temperature and interannual variation in precipitation on flowering date within the range of *E. californica*. These analyses provide one of the first assessments of intraspecific variation in phenological sensitivity to climate in Mediterranean environments and underscore the utility and promise of CCH2's herbarium specimen data for addressing novel questions concerning plant phenological responses to climate change.

METHODS

Study System

The California Poppy, *Eschscholzia californica*, is a common and widespread annual or perennial herb that is native to the west coast of North America and has been introduced as a cultivated wildflower across the continent. California Poppies inhabit a wide range of habitats and climates but are most common in open grasslands, hillsides, coastal bluffs, and road cuts, where they co-occur with grasses and other wildflowers. The species is highly morphologically variable and has been variously divided into several taxa, yet all share the reproductive structures characteristic of the taxon: smooth, pointed buds enclosed by a fused, cap-like calyx; flowers with four bright yellow or orange petals; and narrow, cylindrical fruits arising from a swollen, obconic receptacle (Hannan and Clark 2012). An herbarium specimen displaying these features is shown in Fig. 1. We selected *E. californica* for this study because of its distinct and conspicuous reproductive features (which make the phenological status of specimens relatively easy to score), its wide distribution across California, and its iconic status in the state. Furthermore, emerging research suggests that phenological traits of *E. californica* are correlated with fitness and vary among populations occupying different climates (Ryan and Cleland 2019).

Dataset Preparation

We visualized all available and georeferenced California specimen records for *E. californica* using the Map Search feature of the Consortium of California Herbaria CCH2 data portal (2020; <https://cch2.org/>) and identified regions of the state with relatively low sampling. We then georeferenced



FIG. 1. Digitized herbarium specimen of the California Poppy, *Eschscholzia californica*, displaying an unopened flower bud (far right), open flowers (center and far left), and fruits (right, center, and left). Image from UC Davis Herbarium.

additional specimen records from poorly represented counties using GEOLocate (<https://www.geo-locate.org/>) in CCH2 according to CAP TCN protocols (Wieczorek et al. 2012; Pearson 2020; Zermoglio et al. 2020). As a result, 2199 of the 4197 U.S. *E. californica* specimens in CCH2 were georeferenced and thus usable for our analyses. Taxonomic synonyms of *E. californica* (see Appendix S1) were included in this search and treated as *E. californica* specimens for all analyses. We then used the Trait Coding from Images tool in CCH2 to score the phenological status of all *E. californica* specimens that had precise date and location information but that had not previously been scored by other efforts (see ‘Phenological data’ section below). Excluding specimens without precise date information reduced the dataset to 2101 specimens.

We downloaded the complete specimen records for the 2101 dated and georeferenced herbarium specimens of *E. californica* in CCH2 as of November 10, 2020. To download phenological data along with other available specimen data, we selected the “include Occurrence Trait Attributes” option when downloading the records from the CCH2 public search. This download results in a Darwin Core “MeasurementOrFact” extension file (<https://dwc.tdwg.org/terms/#measurementorfact>) that is separate from the main occurrences table. Each line in the table corresponds to one phenological attribute (e.g., Open Flower present) that then corresponds to a specimen, such that several lines in the table may correspond to the same specimen. To merge the phenological data with the occurrences data in a more intuitive format, we used custom R code (R Core Team, R Foundation for Statistical Computing, Vienna, Austria) developed by the California Phenology Network (<http://doi.org/10.5281/zenodo.4298817>).

We used the *tidyverse* package in R (Wickham et al. 2019) to filter the merged specimen and phenological data, excluding all specimens that lacked a phenological score (see ‘Phenological data’). We also removed specimens collected after day of year (DOY) 250 (September 7th) because we were primarily interested in factors influencing spring and summer flowering dates, and we suspected that flowering specimens collected after DOY 250 may have been either erroneously scored or collected from sites at which the plants were responding to disturbance, cultivation, or other non-seasonal cues. The resulting dataset consisted of 993 specimens.

Phenological data. The phenological scores of angiosperm specimens in CCH2 are coded according to the first- and second-order questions outlined by Yost et al. (2018). The possible first-order scores are “reproductive,” “sterile,” or “not scorable.” Second-order phenological scores reflect the presence or absence of four reproductive structures: unopen flowers, open flowers, senesced flowers, or fruits. Specimens with second-order scores that indicate the

presence of any reproductive structure are automatically assigned a first-order score of “reproductive” as well.

Phenological scores in the Trait Attributes Table in CCH2 have primarily been produced by one of two methods: (1) visual inspection of specimens or specimen images or (2) text mining of label data. Visual inspection and scoring of specimen images method (1) is facilitated by the Trait Coding from Images tool in CCH2, which presents the user with images of specimens from any user-selected taxon. The user then selects the appropriate score(s) (i.e., presence or absence of reproductive structures) from a list of options displayed next to the specimen image. Phenological scores for *E. californica* specimens produced using this method were created by trained student technicians and staff.

For method (2), the text mining tool in CCH2 was used to view textual data from the labels of herbarium specimens and, when possible, to score the stated or implied presence of reproductive structures, as indicated by the text. For example, specimens with label text including “flowers red” would be scored as “open flower present.” Descriptions were interpreted conservatively; for instance, if a description included the phrase “red-flowered annual,” no phenological score was assigned because it was unclear whether the note refers to the species in general or the specimen itself. A screenshot of this tool is shown in Appendix S2.

We restricted our dataset to include only specimens scored as “open flowers present.” Although finer-scale methods of scoring the phenological status of herbarium specimens have been devised (Love et al. 2019; Pearson 2019a), prior research indicates that the coarse, presence/absence scoring schema is effective for identifying climatic trends given the large size of our dataset (Pearson 2019a). The resulting dataset consisted of 993 specimens, collected from 1901 to 2019, with high geographic variation in sampling location (Fig. 2).

Climate data. We calculated long-term temperature and precipitation conditions in each collection site, and temperature and precipitation “anomalies”—the difference between climatic conditions in the year of specimen collection (as well as the previous year) and long-term mean temperature and precipitation for that site. To do so, we obtained monthly time series of mean temperatures and cumulative precipitation (hereafter ‘T_{ave}’ and ‘PPT’, respectively) from January of 1901 to December of 2019 for each site of specimen collection in our sample using ClimateNA v.6.30 (Wang et al. 2016). ClimateNA is a software package that interpolates gridded data from PRISM (PRISM Climate Group, Oregon State University, <http://prism.oregonstate.edu>) at a 4 km × 4 km resolution to produce scale-free climatic estimates adjusted for elevation. We calculated long-term climatic conditions by averaging mean annual temperatures (MAT) and cumulative annual

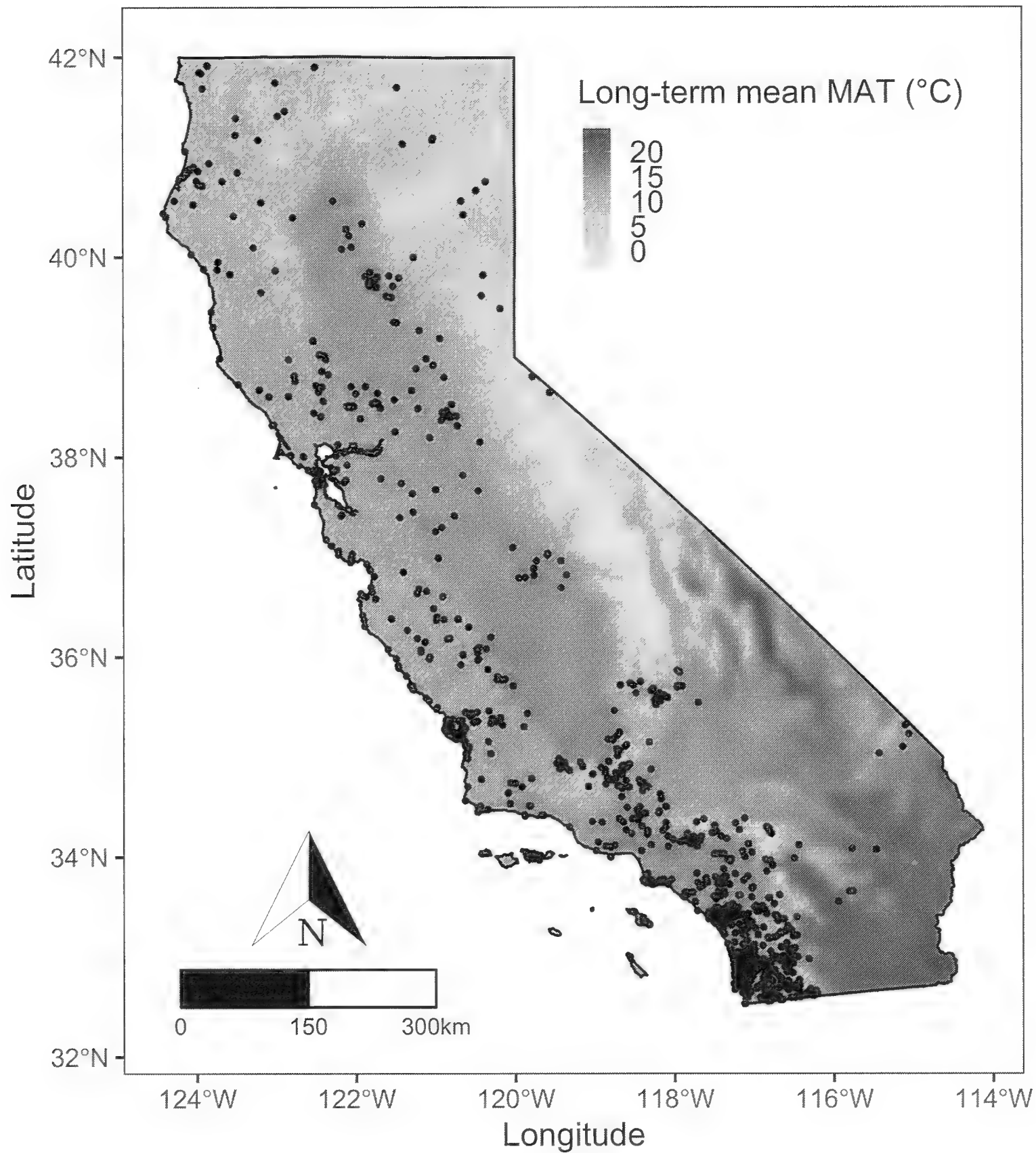


FIG. 2. Collection sites of the 993 *E. californica* specimens in our cleaned dataset. The shading gradient indicates long-term mean annual temperature (MAT) from 1901 to 2019 (see ‘Climate data’ for calculation of long-term MAT).

precipitation (MAP) at each site of specimen collection throughout the study period (1901 to 2019). Similarly, for each collection site, we calculated long-term T_{ave} and PPT for each calendar month (Jan–Dec) by averaging observed conditions for a given month between 1901 and 2019 (the full range of available climate data). We calculated long-term means over the full time range of the data to account for all climatic variability in our estimates of mean climate, but using a shorter, pre-warming window (1940–1970) generated nearly identical estimates. This yielded 120-year averages for MAT and MAP (hereafter ‘long-term MAT’ and ‘long-term MAP’, respectively), and 120-year averages for T_{ave} and PPT for every calendar month (hereafter ‘long-term T_{ave} or PPT’) in each collection site. For each specimen in our sample, we calculated monthly climate deviations (hereafter ‘anomalies’) from long-term conditions in its year and site of collection, and in the previous year, as the difference between observed T_{ave} or PPT and long-term conditions for that month. This yielded 24 monthly climate anomalies (12 monthly anomalies in the year of collection, and 12 anomalies in the previous year) for both T_{ave} and PPT for each specimen in our sample.

No monthly anomaly was significantly correlated with its corresponding long-term mean ($|r| < 0.31$ for all months), indicating that multicollinearity did not affect our results.

Locations of specimen collection varied widely in long-term MAP conditions, with mean annual cumulative precipitation ranging from 220 mm to 1422 mm for the central 95% of the long-term MAP distribution. Consequently, the biological effects of net PPT anomalies of equal magnitude are likely to vary among sites of specimen collection because their magnitude in proportion to local mean precipitation conditions may be vastly different. To account for this, for each collection site, we calculated precipitation anomalies in each month in the year of collection, and the previous year, proportional to long-term MAP for that month. The resulting proportional PPT anomalies are expressed as a fraction of long-term MAP; a proportional PPT anomaly equal to 1 is of magnitude equal to the long-term MAP for that seasonal window in that site, whereas a value of 0.5 would correspond to an anomaly of 50% of the magnitude of long-term MAP for that period.

Analyses

Identifying phenologically influential seasonal windows. We identified the seasons during which PPT and T_{ave} influenced flowering date most strongly using a sliding window analysis, separately estimating phenological sensitivity of flowering DOY (henceforth “flowering date”) to these climate variables in multiple time periods while controlling for variation in long-term MAP and in long-term MAT among sites. For each specimen in our sample, we used T_{ave} and proportional PPT anomalies for each month (from September of the year prior to collection to May of its year of collection) as predictor variables. To obtain anomalies for broader time windows, we averaged monthly anomalies for successive 2-month (Sep–Oct, Oct–Nov, Nov–Dec, etc.) and 3-month periods (Sep–Oct–Nov, Oct–Nov–Dec, etc.). Then, we estimated the effects of PPT and T_{ave} anomalies on flowering date in each time period by fitting a multiple regression for each time window and each climate variable. For each regression, we used the collection date of each specimen in year i and site j as a response (DOY_{ij}), long-term MAP and long-term MAT in each site j as controls (*Long term MAP_j* and *Long term MAT_j*, respectively), and either T_{ave} or proportional PPT anomalies in year j and seasonal window k (1-month, 2-month, or 3-month interval) as a predictor ($T_{ave} \text{ anomaly}_{jk}$ or $PPT \text{ anomaly}_{jk}$) (Equation 1).

Equation 1

$$DOY_{ij} = \beta_1 \times \text{Long-term MAP}_j \\ + \beta_2 \times \text{Long-term MAT}_j \\ + \beta_3 \times T_{ave} \text{ or PPT anomaly}_{ijk} + \varepsilon$$

Among time-windows for which climate anomalies produced statistically significant coefficients, those of greatest magnitude were interpreted as affecting flowering date most strongly in *E. californica*. Changes in the magnitude, direction, and significance of climatic parameters over consecutive time windows (e.g., shifts from negative to positive PPT coefficients from Fall to Spring months) were consistent among 1-month, 2-month, and 3-month windows. However, models including climate variables averaged over 2-month windows showed better fit (i.e., higher model R^2 values) and lower standard errors in parameter estimation (data not shown). Consequently, we report results for time window analyses using 2-month periods.

Phenological sensitivity to long-term and interannual climate variation. To assess how variation in long-term climatic conditions and interannual climate variation influence the flowering date of *E. californica*, we evaluated the effects of T_{ave} and proportional PPT anomalies and long-term MAP and long-term MAT conditions on flowering date among collection sites. We selected the 2-month windows for T_{ave} and proportional PPT anomalies showing the greatest effects on flowering date. Some

periods that showed significant effects for T_{ave} and proportional PPT anomalies were moderately or highly correlated. Consequently, for both proportional PPT and T_{ave} anomalies, we included more than one period only if (1) their correlation coefficients were less than or equal to 0.6, (2) inclusion of the period with an effect of lesser magnitude yielded a significant regression coefficient, and (3) inclusion of the additional period lowered the AIC value of the model by two or more.

To evaluate whether the effects of interannual variation in precipitation and temperature (i.e., anomalies) varied among parts of the range of *E. californica* with different long-term climatic conditions, we tested whether T_{ave} and proportional PPT anomalies significantly interacted with long-term MAT and MAP across specimen collection sites. We fit multiple linear regression models including interaction terms for either long-term MAT or MAP and T_{ave} or proportional PPT anomalies (i.e., MAT normals| T_{ave} anomalies, MAT normals|PPT anomalies, MAP normals| T_{ave} anomalies, and MAP normals|PPT anomalies for each 2-month window selected for inclusion in the final model). We only included one interaction term per regression model, and from these models we selected significant interaction terms for inclusion in the final model reported in this study. The final regression included flowering date for each specimen as a response (DOY_{ij}), long-term MAT and long-term MAP (*long-term MAT_j* and *long-term MAT_j*), April–May T_{ave} anomalies (*Apr–May T_{ave} ij*), February–March proportional PPT anomalies (*Feb–Mar PPT_{ij}*), October–November proportional PPT anomalies in the year (*Oct–Nov PPT_{ij}*), and the interaction between April–May T_{ave} anomalies and long-term MAT (*long-term MAT_j \times Apr–May T_{ave} ij*) as predictors (Equation 2).

Equation 2

$$DOY_{ij} = \beta_1 \times \text{Long-term MAT}_j \\ + \beta_2 \times \text{Long-term MAP}_j \\ + \beta_3 \times \text{Apr–May } T_{ave}_{ij} \\ + \beta_4 \times \text{Oct–Nov PPT}_{ij} \\ + \beta_5 \times \text{Feb–Mar PPT}_{ij} + \beta_6 \\ \times \text{MAT Long-term}_j \\ \times T_{ave} \text{ Apr–May}_{ij} + \varepsilon$$

All predictors were mean-centered for easier biological interpretation of the intercept and main effects of the model. To compare the magnitude of predictor effects relative to their scale of variation, we fit the same model, scaling all predictors to a standard deviation of 1. By doing so, regression coefficients can be interpreted as the expected change in the response (flowering date as DOY) for a change of one standard deviation (SD) in each predictor. Variance inflation factors (VIFs) for all predictors

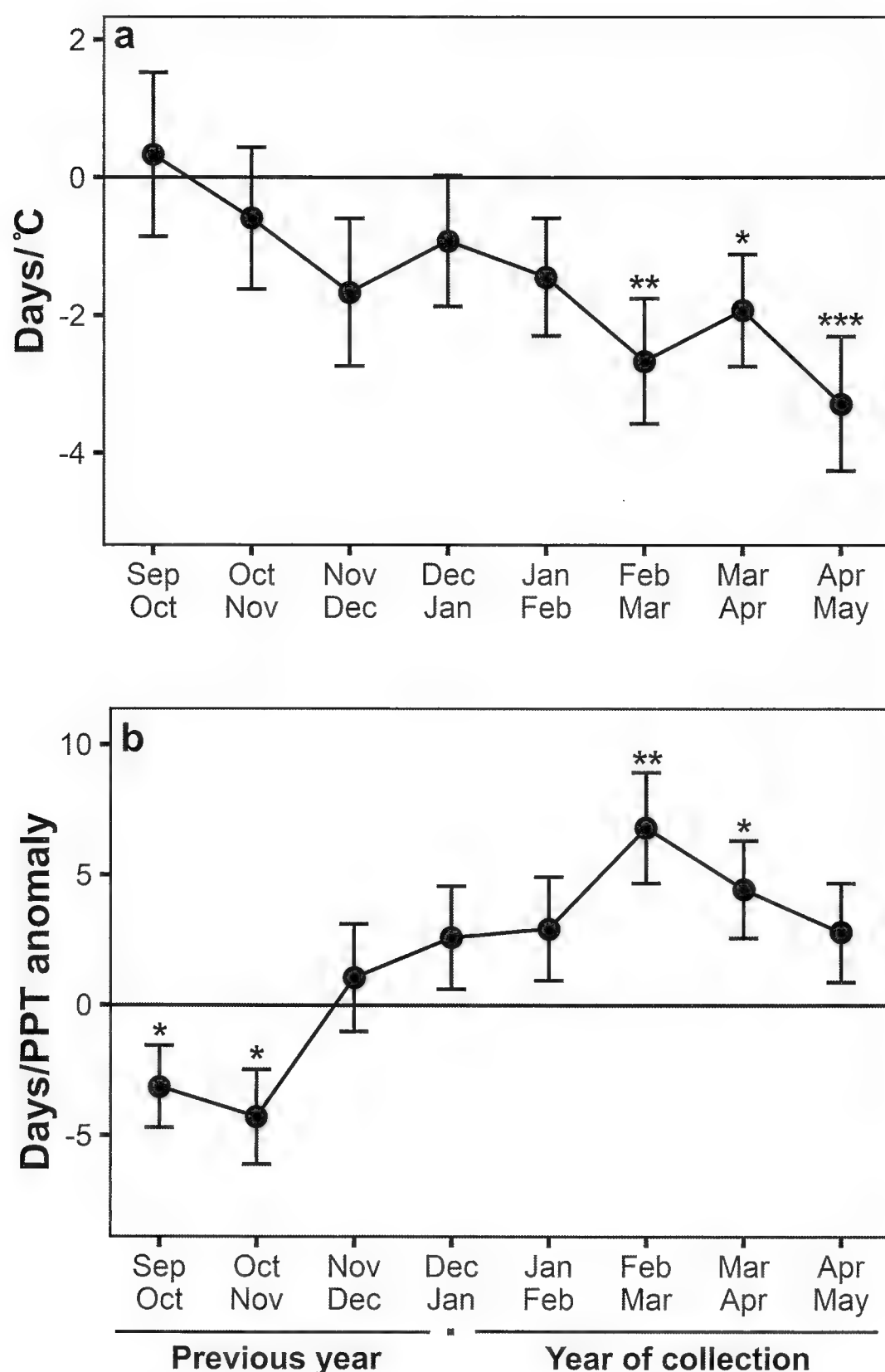


FIG. 3. Sensitivity of flowering phenology to interannual variation in (a) mean temperature anomalies and (b) mean cumulative precipitation anomalies (proportional to mean precipitation in that period within each site) for different 2-month windows prior to the mean month of flowering of 993 herbarium specimens of *E. californica*. Asterisks represent significant phenological sensitivities to temperature or proportional precipitation anomalies for the focal 2-month period at $\alpha = 0.05$ (*), $\alpha = 0.01$ (**), and $\alpha = 0.001$ (***).

were lower than 1.34, suggesting collinearity was unlikely to cause estimation problems.

Temporal trends in climate. We quantified temporal trends in the climate variables most strongly influencing flowering date in *E. californica* using generalized additive models implemented using the ‘mgcv 1.8-31’ package in R (GAMs; Wood, S. and M. S. Wood. version 1, 29). These models used T_{ave} and proportional PPT anomalies as response variables (see Equation 2), year as a predictor, and long-term MAT and long-term MAP as covariates controlling for potential geographic variation in the locations of specimen collection over time. To assess whether the degree of climatic change varied throughout the range of *E. californica*, we repeated the analysis using linear regressions and included interaction terms between year and long-term MAT

and long-term MAP. We found no significant interactions and report results only from the GAMs.

Temporal trends in phenology. We used multiple linear regression to assess whether flowering dates of *E. californica* have changed over time, and to evaluate whether rates of phenological change have varied among regions with different long-term climatic conditions within the species’ range. Changes in the regions where specimens are sampled over time could lead to spurious temporal trends in phenology (e.g., recently collected specimens may come from warmer areas where flowering occurs earlier, on average, even if flowering dates have not changed over time); therefore, we included long-term MAP and long-term MAT as controls. To evaluate whether the magnitude of phenological change varies throughout the range of *E. californica*, we included interaction terms of year with long-term MAT and year with long-term MAP in each collection site, retaining only significant interaction terms in the final model. The resulting regression included flowering dates as a response (DOY_{ij}), year as a predictor ($year_i$), long-term MAT and MAP conditions as controls ($Long-term MAT_j$ and $Long-term MAP_j$), and an interaction term between long-term MAT and year of collection ($Long-term MAT_j \times year_i$).

Equation 3

$$DOY_{ij} = \beta_1 \times Long-term MAT_j + \beta_2 \times Long-term MAP_j + \beta_3 \times Year_i + \beta_4 \times Long-term MAT_j \times Year_i + \varepsilon$$

We tested for temporal and spatial autocorrelation in the residuals of all models using spatial and temporal distance matrix regressions, but found no evidence for either ($R^2 \leq 0.001$ and $P \geq 0.07$ for all tests). Variance inflation factors (VIFs) for all predictors were lower than 1.29. All analyses were conducted using R v4.0.3 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Interaction plots were visualized using the *visreg* package v2.7.0 (Breheny and Burchett 2017) and *ggplot2* v3.3.2 (Wickham 2016).

Data Availability

The authors have made the cleaned specimen dataset and analysis code available via a public, online repository (<http://doi.org/10.5281/zenodo.4383254>).

RESULTS

Seasonal Phenological Sensitivities

The magnitude of phenological sensitivity of *E. californica* to temperature gradually increased from the fall of the year before flowering to the spring of the flowering year (Fig. 3a), with higher temperature anomalies associated with earlier flowering dates. We detected significant negative effects of temperature

TABLE 1. RESULTS OF LINEAR REGRESSION OF FLOWERING DATE AND CLIMATE VARIABLES. Output of linear regression of day of year of flowering (DOY) vs. long-term mean cumulative precipitation (MAP) and mean annual temperature (MAT) at each site of collection, and cumulative precipitation and mean temperature anomalies for various 2-month periods in the year and site of specimen collection (See Equation 2 in Methods Section; adjusted $R^2 = 0.21$, $df = 986$, $F = 45.9$, $P < 0.001$). All predictors in the model were mean-centered and standardized to a SD of one to facilitate comparison of effect sizes among predictor variables. Negative coefficients (Coef.) indicate advancement in DOY (earlier flowering), while positive coefficients indicate delays in DOY (later flowering).

Variable	Coef.	SE	<i>t</i>	P
Intercept	116.40	1.09	106.34	< 0.001
Long-term MAP	2.58	1.26	2.05	0.041
Long-term MAT	-15.27	1.25	-12.17	< 0.001
Apr–May T° anomaly	-2.91	1.13	-2.58	0.010
Feb–Mar PPT anomaly	4.01	1.16	3.46	< 0.001
Oct–Nov PPT anomaly	-3.67	1.12	-3.26	0.001
Long-term MAT × Apr–May T° anomaly	2.84	0.96	2.94	0.003

anomalies on flowering date (as DOY) for periods spanning the end of winter and most of the spring in the year of flowering, indicating earlier flowering in warmer-than-average years by 2.7–3.3 days/°C (Fig. 3a).

We detected significant sensitivity to cumulative precipitation anomalies (proportional to long-term cumulative precipitation in each collection site) in both the fall of the year prior to flowering and the spring of the flowering year (Fig. 3b); however, proportional precipitation anomalies during these periods had opposite effects on flowering date, with greater-than-average September–October and October–November precipitation leading to advances in flowering date (−3.1 and −4.3 days per proportional PPT anomaly, respectively), and greater-than-average February–March and March–April precipitation resulting in delays in flowering date (6.8 days and 4.4 days per proportional PPT anomaly, respectively).

Phenological Sensitivity to Long-term and Interannual Climate Variation

Our model (Equation 2) explained 21% of the variation in flowering date among specimens (Table 1). We found significant relationships between long-term climatic conditions among sites of specimen collection and flowering date, with geographic gradients across increasing MAP associated with later flowering dates (0.8 days/100 mm; Fig. 4a), and geographic gradients across increasing MAT associated with earlier flowering dates (−7.2 days/°C; Fig. 4b). Among collection sites represented in our data, variation in standardized long-term MAT was associated with greater changes in flowering date than variation in standardized long-term MAP; changes in long-term MAT of one SD (2.0 °C) among collection sites were associated with average advances in flowering date of 15 days, while changes in long-term MAP of one SD (305 mL) were associated with average delays in flowering date of 2.6 days (Table 1, Figs. 4a and b).

The opposing effects on flowering date of proportional PPT anomalies for October–November and

February–March revealed through sliding-window analyses remained consistent in magnitude and direction when both proportional PPT anomalies were included in the same model (see Equation 2). October–November and February–March proportional PPT anomalies had effects on flowering date of similar magnitude (but opposite in direction) per one SD of change in each variable (Table 1, Fig. 4c and d).

We detected a significant interaction between long-term MAT among sites of specimen collection and April–May T_{ave} anomalies, indicating that the effects of interannual temperature variation on flowering date vary with long-term temperature conditions (Fig. 5). A difference of 2.4°C in long-term MAT relative to the mean among sites was associated with changes of ±3.0 days/°C in the effects of April–May temperature anomalies on flowering date (SE = ±0.96 days/°C). Accordingly, the phenological sensitivity to April–May temperature anomalies among *E. californica* specimens collected in relatively cold regions (2.4 °C lower than average, or 10th percentile) was −5.5 days/°C, whereas that of specimens collected in relatively warm regions (1.8 °C higher than average, or 90th percentile) was only −0.3 days/°C. The phenological sensitivity of *E. californica* to April–May temperatures and to October–November and February–March precipitation did not significantly interact with long-term MAP. Consequently, interannual temperature variation had greater effects on flowering date than interannual precipitation variation in colder-than-average sites, whereas precipitation had a greater effect on flowering date than temperature in warmer-than-average sites. For example, in relatively warm regions (1.8°C greater than average), an increase of 1.1°C (1 SD given the range of our data) in April–May temperatures was expected to result in a flowering advancement of only 0.4 days, whereas increases of 52% and 61% (1 SD given the range of our data) in fall and spring precipitation relative to long-term MAP in each collection site, which did not vary with either long-term temperature or precipitation, were associated with flowering advances of 3.7 days and delays of 4.0

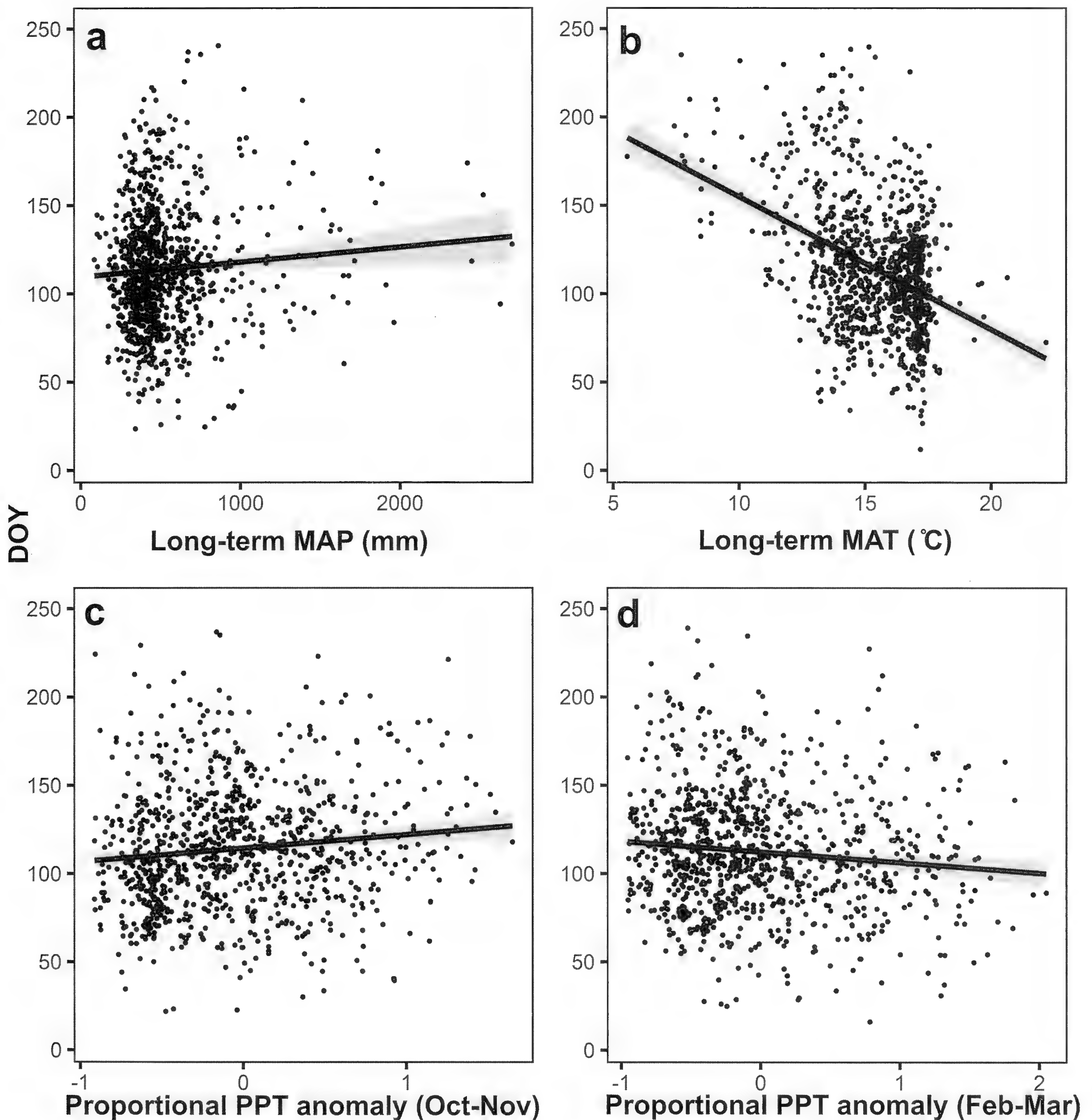


FIG. 4. Relationship between date of flowering (DOY) among 993 herbarium specimens of *E. californica* and (a) long-term mean MAP at the site of specimen collection (b) long-term mean MAT at the site of specimen collection (c) anomalies in cumulative February–March precipitation in the year of collection proportional to long-term averages for the site of collection, and (d) anomalies in cumulative October–November precipitation in the year of collection proportional to long-term averages for the site of collection.

days, respectively (Table 1). These results were consistent when using temperature anomalies calculated over other significant two month periods (February–March or March–April; results not shown).

Climate Change Over Time

We detected significant increases in T_{ave} anomalies over time among the 993 collection locations represented in our sample, with an increase of

approximately 1°C in mean April–May temperature from 1970 to 2019 (i.e., ~ 0.2 °C/decade; $P < 0.001$; Fig. 6a). We detected a significant reduction in October–November proportional PPT anomalies over time among collection sites ($P = 0.002$; Fig. 6b). However, the reduction in proportional PPT anomalies between 1970 and 2019, the period best represented in our sample and that marks the onset of rapid climate change, was very small relative to the range of variation in the data; decreases in PPT anomalies proportional to long-term MAP in each

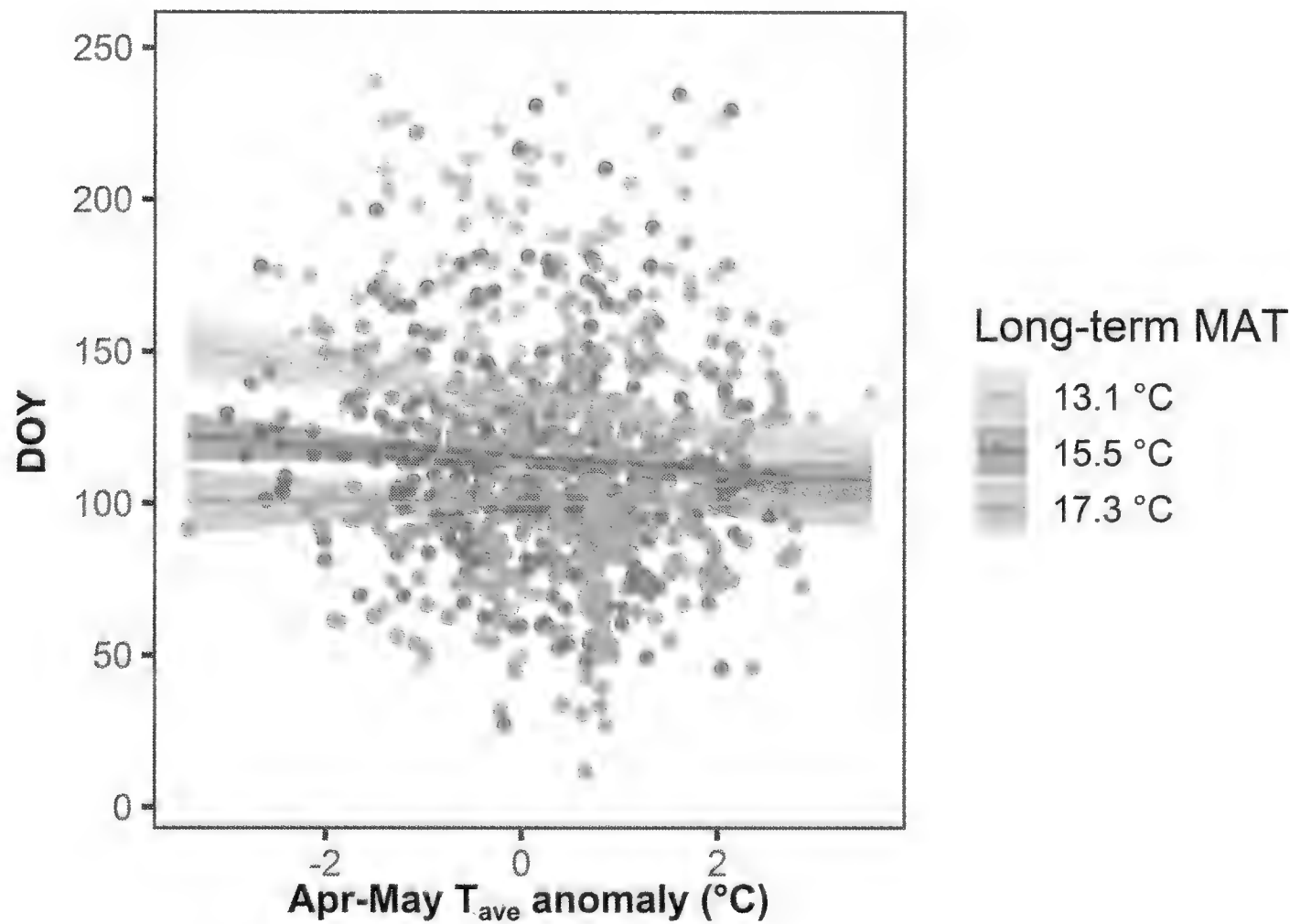


FIG. 5. Relationship between date of flowering (DOY) and mean April-May temperature anomalies across portions of the range of *E. californica* differing in long-term mean annual temperature (MAT). Red points and lines designate specimens in regions with warmer-than-average long-term MAT, green points and lines designate regions with approximately average long-term MAT, and blue points and lines designate specimens in regions with cooler-than-average long-term MAT.

site since 1970 were lower than 0.1, corresponding to a reduction of less than 10% of the mean October–November cumulative precipitation per site, whereas mean interannual variation was 0.52, or 52% of October–November long-term MAP in a site. We found no significant temporal trends in February–March proportional PPT anomalies among specimen collection sites (Fig. 6c).

Temporal Trends in Flowering Date

Our model designed to detect temporal trends in *E. californica* flowering date (Equation 3) revealed an average advancement of flowering date of 0.8 days per decade ($P = 0.048$). However, we detected a significant interaction between long-term MAT and the effect of year, indicating that rates of phenological change over time across the range of *E. californica* depended on local long-term temperatures (Fig. 7, $P = 0.002$). A difference of 2.4°C in long-term MAT relative to the mean among sites was associated with changes of ± 1.4 days per decade in the rate of flowering date change over time. Accordingly, specimens collected in colder-than-average regions (2.4 °C lower than average; 10th percentile) were predicted to advance their flowering phenology at a rate of 2.6 days per decade, whereas specimens collected in relatively warm regions (1.8 °C greater than average; 90th percentile) were predicted to delay flowering at a rate of only 0.4 days per decade.

DISCUSSION

Differences in long-term temperature and precipitation were associated with differences in flowering date, with later flowering at sites with relatively high

precipitation, and earlier flowering at warmer-than-average sites. Furthermore, flowering date variation depended more strongly on long-term mean temperature than on long-term mean precipitation. Interannual variation (i.e., anomalies) in both temperature and precipitation influenced flowering date, but this sensitivity depended on long-term temperature at the collection site, with greater sensitivity to temperature anomalies in colder regions. Consistent with observed temporal warming trends across the range of *E. californica*, we detected an advancement of flowering date over time that mirrors the long-term temperature-dependence of phenological sensitivity to changes in temperature; the rate of change of flowering date in cooler-than-average sites has outpaced that of warmer-than-average sites. This intraspecific variation in phenological sensitivities across the range of *E. californica*, along with regional differences in projected climate change, may result in complex phenological changes for *E. californica* that may impact fitness and species interactions within herbaceous forb and grassland communities. The spatiotemporal coverage of our data, enabled by the digitization of herbarium specimens, provides a nuanced view of range-wide phenological trends that may inform future studies designed to distinguish the mechanisms and effects of phenological shifts.

Phenological Sensitivities of Flowering Date to Seasonal, Interannual Variation

Of the interannual seasonal temperature variables investigated in this study, late-winter and spring (Feb–May) temperature anomalies in the year of flowering had the strongest effects on flowering date

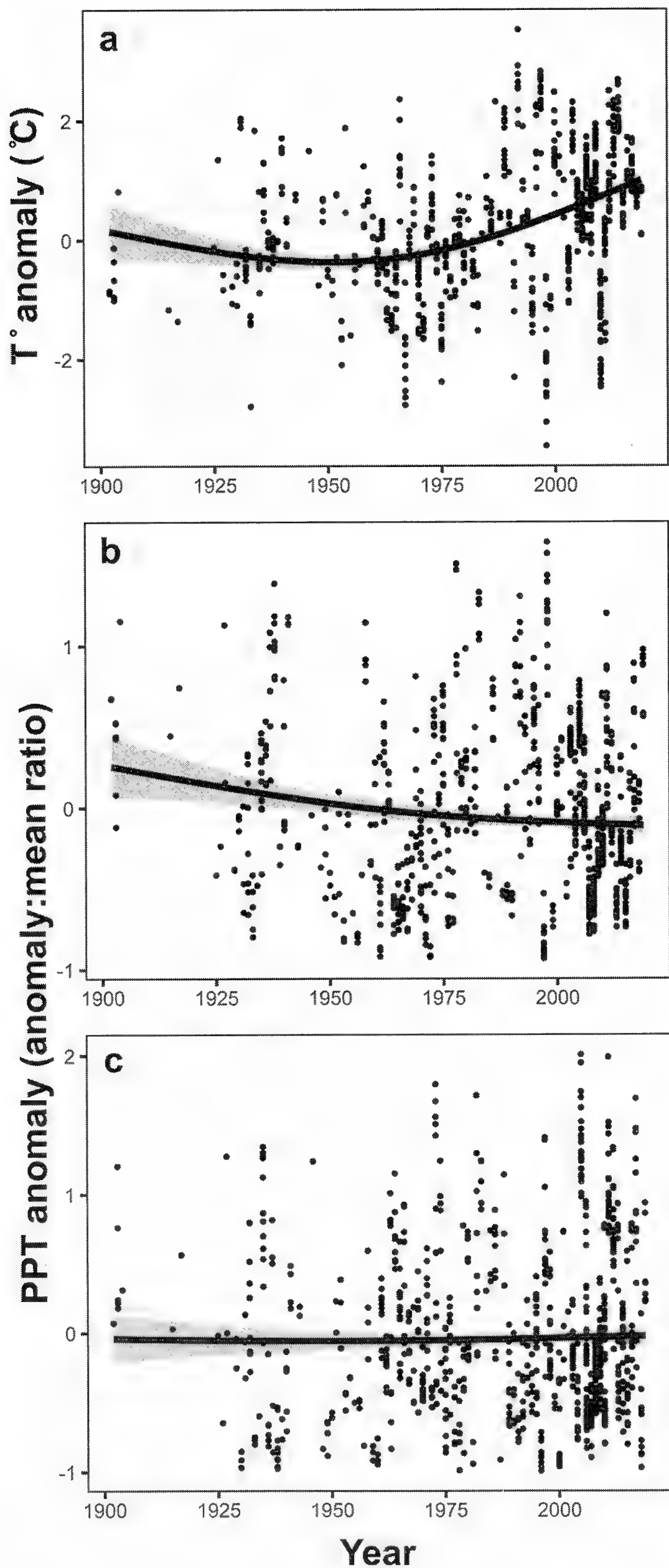


FIG. 6. Change over time in (a) April-May temperature anomaly ($^{\circ}\text{C}$), (b) February-March cumulative precipitation anomaly (proportional to long-term averages for the site), and (c) October-November cumulative precipitation anomaly (proportional to long-term averages for the site) across 993 sites of specimen collection throughout the range of *E. californica*.

of *E. californica*. Increases in temperature advanced flowering at a rate of 2.7–3.3 days per 1°C increase in spring temperature anomaly (Fig. 3a). Fall and winter temperature anomalies in the year prior to

flowering did not significantly affect flowering date. This may reflect a short or nonexistent vernalization requirement in *E. californica*, which could be expected for plants in Mediterranean climates that are unlikely to experience very cold winters. The advancing effect of spring temperature anomalies on flowering date is consistent with estimates of flowering advancements in other climates and for other taxa (Miller-Rushing and Primack 2008; Willis et al. 2017; Pearson 2019b). Considering the warming trends that we observed across the range of *E. californica* (Fig. 6) and projections of future climate change (Bedsworth et al. 2018), flowering dates of *E. californica* are projected to continue to advance.

However, our analyses also revealed that phenological sensitivities to interannual temperature variation depended on long-term temperature at the site of collection; *E. californica* in cooler-than-average sites were more responsive to spring temperature than those in warmer-than-average sites. This site-dependence resulted in phenological shifts of several days per $^{\circ}\text{C}$ in colder-than-average locations, and in an apparent lack of phenological responsiveness to temperature in warmer-than-average locations. Few herbarium-based studies have quantified intraspecific variation in phenological sensitivity to climate and of phenological change over time across the range of a single species (but see Matthews and Mazer 2016; Munson and Long 2017; Song et al. 2020, 2021). Our results demonstrate that, for a single species, estimates of phenological sensitivity derived from data in a single location might not accurately predict phenological sensitivities to climate in other locations throughout the range of the species. Moreover, patterns of intraspecific variation may differ among taxa, making generalization difficult. For example, Song et al. (2020) discovered greater phenological sensitivity of a perennial herb in dry, warm climates, in contrast to our findings. The mechanisms underlying the opposite trend in *E. californica* are unknown but may be related to developmental limits; California Poppies in warmer-than-average locations may already be flowering as early as developmentally possible, and flowering any earlier might mean doing so at such a small size that the amount of stored resources is insufficient to ensure fruit maturation. Alternatively, flowering earlier could result in phenological mismatches with their pollinators. If either scenario applies, then poppies in warmer climates may face particular challenges with future climate change. If poppies are unable to shift flowering into cooler months or otherwise adapt to environmental stressors, extreme heat or drought during flowering and fruit development could increase mortality and decrease individual fitness by decreasing reproductive output (Cox et al. 2021). In these circumstances, the distribution of *E. californica* may shift toward cooler regions at higher latitudes and elevations, similar to what has been observed and predicted for other plant taxa in California (Kelly and Goulden 2008; Ackerly et al. 2020).

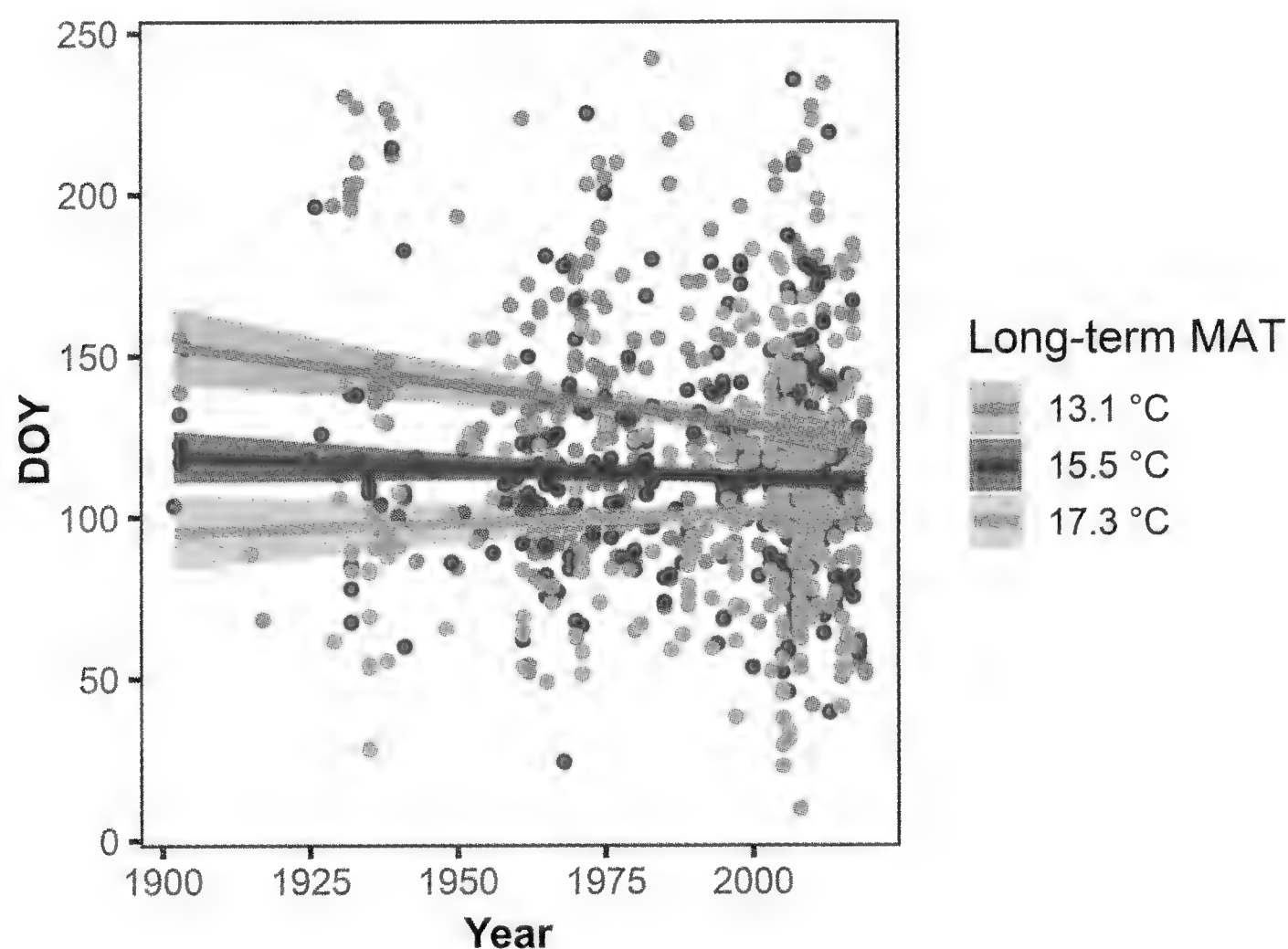


FIG. 7. Temporal trend in the flowering phenology of *E. californica* across portions of its range differing in long-term mean annual temperature conditions. Red points and lines designate specimens in regions with warmer-than-average long-term MAT, green points and lines designate regions with approximately average long-term MAT, and blue points and lines designate specimens in regions with cooler-than-average long-term MAT.

Predicting flowering date of *E. californica* in response to climate conditions is further complicated by the opposing effects of proportional cumulative precipitation anomalies in different seasonal windows. Flowering date was significantly delayed in response to increased spring (Feb–Apr) proportional PPT anomalies, but advanced with increased fall (Oct–Nov) proportional PPT anomalies in the year prior to flowering (Fig. 3b). Previous research has discovered contrasting effects of seasonal temperatures on phenological events (Cook et al. 2012; Hart et al. 2014; Pearson 2019b); however, to our knowledge, differences in the direction of the effects of precipitation for different seasons have seldom been reported (but see Munson and Sher 2015). Precipitation has commonly been found to have only a delaying or non-significant effect on flowering date (Abu-Asab et al. 2001; Matthews and Mazer 2016; Pearson 2019b). In contrast, interannual variation in both fall and spring precipitation influence flowering date in *E. californica*. Increases in fall precipitation might spur earlier germination (for annual individuals) and accelerate vegetative growth of *E. californica* (for both annual and biennial individuals), ultimately advancing spring flowering dates. However, higher-than-average precipitation in the spring may decrease the activity of pollinators (Lawson and Rands 2019) and extend the duration of the growing season; consequently, *E. californica* might have evolved to delay reproductive onset in springs with relatively high precipitation, potentially resulting in greater vegetative growth and resource accumulation prior to flowering.

Relative Influence of Temperature Versus Precipitation on Flowering Date

Although our analyses found that interannual variation in precipitation affected flowering dates, long-term mean temperature was the strongest predictor of flowering date, strongly outweighing the effects of long-term mean precipitation (Fig. 4, Table 1). This finding is consistent with previous research in Mediterranean (Gordo and Sanz 2010) and other climates (Abu-Asab et al. 2001; Hart et al. 2014; Munson and Long 2017), yet differs from other studies in the western U.S. (Matthews and Mazer 2016). Given the extreme water limitation in some areas of the range of *E. californica* and the potential effects of this limitation on fitness and natural selection (e.g., Franks et al. 2007), an overall lower impact of precipitation on flowering dates is somewhat unexpected. It is possible that, rather than having strong effects on flowering date, precipitation affects the duration of flowering and, consequently, total flower, fruit, and seed production. The causal relationships between flowering date and long-term temperature and precipitation are difficult to identify in observational studies. The relationships reported here may reflect adaptation to local climate conditions or they may reflect evolutionary or plastic responses to other environmental factors that may co-vary with climate, such as nutrient availability, pollinator abundances, and exposure to herbivores. Our analyses cannot distinguish between adaptive and plastic phenological responses to climate variation.

Although long-term temperature was consistently more predictive of flowering date than long-term

precipitation, the effects of temperature and proportional precipitation anomalies on flowering date depended on long-term temperature at the collection site. Temperature anomalies had a greater effect on flowering date in colder-than-average sites, whereas precipitation had a greater effect on flowering date in warmer-than-average sites. This indicates that water limitation may have played an important role in shaping the phenological sensitivities of *E. californica* to interannual climate variation, and water availability will likely shape the phenological impacts of future climate change. Given the species- and region-specific effects of climate on phenological events (Petrauski et al. 2019; Song et al. 2020), more research is needed to investigate the relative importance of temperature and precipitation on California plants.

Temporal Trends: the Combined Effects of Phenological Responses to Long-term and Interannual Variation in Climate

Together, the contrasting and context-specific phenological responses of *E. californica* to temperature and precipitation, coupled with significant climate change across the range of the species (Fig. 6), forecast complex future phenological changes for *E. californica*. Our analyses suggest that intraspecific variation in phenological sensitivity has already caused disparate temporal shifts in flowering dates across the range of *E. californica*; flowering dates in cooler-than-average sites have shifted an estimated 3.1 days earlier per decade between 1901 and 2019, while flowering dates in warmer-than-average sites have shifted only an estimated 0.1 days per decade over the same timeframe. It is important to note that, had we not estimated the independent and interacting effects of long-term means and anomalies of temperature and precipitation on flowering date, the magnitude of sensitivity to temperature anomalies and of shifts in flowering date would be, on average, much lower than the sensitivity we identified in cooler-than-average regions of the range of *E. californica*. These results highlight the importance of quantifying intraspecific variation in phenological sensitivity throughout species' ranges, and suggest that estimates of phenological change over time based on data from single locations should be interpreted with caution. Likewise, our results show that the flowering date of *E. californica* is sensitive to interannual variation in both temperature and precipitation, and, for precipitation, variation in two different seasons (fall and spring) had opposing effects on flowering date. Because each of these variables had unique and sometimes opposing effects on flowering date, the net effect in some climatic conditions could be no change in flowering date even though the species is responsive to both temperature and precipitation. As a result, species that appear to be phenologically "insensitive" to past climatic conditions may not be so in all climatic conditions

in the future (Hart et al. 2014), especially as changes in temperature and precipitation may have complex effects on soil moisture. This highlights the importance of large, spatiotemporally diverse phenological datasets such as those afforded by digitized herbarium specimens to tease apart intraspecific and spatiotemporal variation in the phenological effects of climate change.

Limitations and Strengths of Specimen-based Phenological Analyses

Specimen-based phenological data, and thus the analyses presented here, are subject to certain limitations. Herbarium specimens are often collected opportunistically rather than systematically and may therefore contain spatiotemporal and taxonomic biases, such as over-collection near populated places and roads (Dodd et al. 2016; Daru et al. 2017), which could cause under-representation of certain environmental conditions in our data. For example, 42% of specimens in our dataset were collected in the southernmost 10% of latitudes. Collectors may also preferentially collect individuals that are in flower even if the majority of the population is not flowering (Willis et al. 2017). These limitations are largely overcome by the size and provenance of the dataset, our dataset represents the combined collecting effort of over 600 collectors across 22 herbaria, and by data cleaning steps to minimize the effects of outliers that originate from collector bias. Specimen-based data, like many data sources, are correlational and can only hint at the mechanisms underlying spatiotemporal changes in phenological events. Further experimental studies are needed to distinguish how context-specific factors, such as functional traits and local microclimates, will affect phenological events in *E. californica* and other taxa across California's diverse landscape.

Our study focused on a single wildflower species in California, the California Poppy, yet this study represents a path forward to understanding the phenological effects of climate change on plant taxa in the biologically and geographically diverse California Floristic Province. With the availability of large phenological datasets from the digitization and subsequent phenological scoring of herbarium sheets, we are poised to investigate more deeply the species- and region-specific effects of climate on plant phenological events. Furthermore, these types of analyses will enable us to ask broader questions about change across the state, such as whether temperature is consistently a stronger predictor of phenological events in annual and perennial herbs across California, whether phenological sensitivity is phylogenetically conserved (Davies et al. 2013; Mazer et al. 2013), and whether phenological sensitivity differs among species occupying or adapted to unique geological conditions such as serpentine soils. The taxonomic, geographic, and temporal scope of digitized herbarium specimens has the

potential to open a new frontier in the study of plant responses to climate change.

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CAUSES AND CORRELATES OF INTERANNUAL VARIATION IN FLOWERING OF *CALOCHORTUS PLUMMERAE* (LILIACEAE)

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ABSTRACT

Calochortus plummerae Greene (Liliaceae) is a mariposa lily that is restricted to five counties in southern California. Although rarely observed in late seral chaparral during most years, it flowers abundantly after fires. In the spring of 2004, a population of *C. plummerae* was discovered in recently burned chaparral on the campus of California State University, San Bernardino. Long-term study plots were established to monitor year-to-year variation in flowering of *C. plummerae*, and to explore potential causes of this variation. We explored the relationship between annual precipitation and flowering of *C. plummerae*. We also examined the relationship between precipitation and growth of the strap leaf. Finally, we tested effects of experimental herbivory (leaf cutting) on current-year flowering. Flowering was monitored for 10 years, and leaf characteristics (leaf size and leaf loss to herbivory) were monitored for six years. During the driest year of our study, no plants flowered, but a substantial number flowered during wet years. Plants also produced narrower leaves during the driest years of the study. Leaf loss (experimental removal of most of a strap leaf) resulted in failure to flower. Overall, flowering between fires appears to be suppressed by factors that reduce current-year carbon gain: drought and leaf loss to herbivory. Although our data on herbivory were too incomplete to assess its importance in driving the dynamics of flowering in years after fire, the remaining two factors we studied (time since fire and annual rainfall) were sufficient to predict the number of *C. plummerae* plants that flowered each year.

Key Words: *Calochortus plummerae*, chaparral, fire, flowering, geophyte, herbivory, precipitation.

Calochortus plummerae Greene (Plummer's Mariposa Lily) is a geophyte of California chaparral that flowers abundantly after fire. It is endemic to five counties in southern California and has a California Rare Plant Rank of 4.2 ("plants of limited distribution; fairly threatened in California"; California Department of Fish and Wildlife 2021). It usually produces a single, basal strap leaf from an underground corm in the spring, which withers before the plant flowers. Flowering, if it occurs, takes place between May and July (Fiedler 2012). In years between fires, *Calochortus* species often remain in a vegetative state, putting up leaves in the spring (e.g., Christensen and Muller 1975; Keeley et al. 2006). Horton and Kraebel (1955), however, suggested that the corms of *C. plummerae* also become dormant as shrub cover increases in the years after fire, and that the plants produce leaves only in exceptionally wet years.

Fire-stimulated flowering is common among geophytes in Mediterranean climates, including that of *C. plummerae*'s range, and flowering of some species is more tightly restricted to post-fire years than that of others (Stone 1951; Horton and Kraebel 1955; Christensen and Muller 1975; Keeley et al. 1981; Le Maitre and Brown 1992; Rundel 1996; Lamont and Downes 2011). Factors that may contribute to this post-fire bloom include reduced competition for resources (light, nutrients, and water), release of soil nutrients by fire, reduced herbivory, and, potentially, stimulatory effects of smoke on flowering (Christensen and Muller 1975; Keeley 1993; Light et al. 2007; Borchert and Tyler 2009). Furthermore, flowering

itself may deplete energy reserves, contributing to low growth rates and low incidence of flowering after the post-fire bloom (Tyler and Borchert 2003; Borchert and Tyler 2009). In years between fires, geophytes may grow vegetatively or their underground organs may become dormant for some period (Keeley and Davis 2007).

In the fall of 2003, multiple wildfires burned a large fraction of *C. plummerae*'s range, providing an opportunity to study plant fate and phenology in the years following fire. The largest of these were the Simi Fire, the Old Fire, the Grand Prix/Padua Fire, which together burned over 105,000 ha across Ventura, Los Angeles, and San Bernardino Counties (Clark et al. 2003). Prolific flowering of *C. plummerae* the following spring made it apparent that the species was not as rare as had previously been believed. Among the newly discovered populations of *C. plummerae* that year was one on the campus of California State University San Bernardino, a campus that partially burned in the Old Fire (Williams et al. 2006).

The primary goals of this study were to quantify changes in flowering of *C. plummerae* in the years following fire, describing the degree to which flowering was restricted to the post-fire environment, and to explore correlates of interannual variation in flowering (in particular, variation in rainfall). Three years into the study, however, we expanded the focus to measure plant survival, potential dormancy, interannual variation in leaf size, and herbivory. We did not measure seedling recruitment because we could not distinguish seedlings of *C. plummerae* from

seedlings of *Calochortus splendens* Douglas ex Benth., which was also present on the site (Williams et al. 2006). Flowering was monitored for 10 years and vegetative characteristics (leaf emergence, leaf size, and leaf loss to herbivory) were monitored for six years. We explored the relationship between annual precipitation and flowering of *C. plummerae* plants, we documented year-to-year variation in the size of the strap leaf produced (specifically, leaf width), and we tested effects of reducing leaf area (experimental herbivory or leaf clipping) on current-year flowering.

METHODS

Flowering, Survival and Dormancy

In June 2004, we established three 5 m × 5 m plots on Badger Hill, an outcrop of Pelona schist surrounded by alluvium (Miller and Matti 2001), on the campus of California State University San Bernardino (34.186°, -117.315°). We sited plots so that each contained a large number of plants of *Calochortus plummerae*. Plots were located along ridges and on northeast-facing slopes, at elevations of 540–565 m. We mapped all individuals of *C. plummerae* in the plots and marked each with a small colored stake located 5 cm to the east of each plant (or in another direction when rocks precluded stake insertion). We censused plants between May and June every year for 10 years to determine if they had produced a flowering stalk that year. New individuals that flowered over the course of the study were also marked and mapped.

In 2007, to better assess plant survival and dormancy, we began censusing plants twice a year: once during the flowering season, as described above, and once earlier in the season (March or April). Leaves were still alive during the earlier census, but generally dead and/or missing during the later census. Censusing twice allowed us to detect plants that produced leaves but did not flower.

Relationship of Flowering to Rainfall and Time Since Fire

We explored the effect of annual precipitation on flowering of *C. plummerae* by using a multiple regression approach that modeled flowering as a function of annual precipitation and time since fire. The number of plants flowering (pooled data from the three plots) was regressed against total precipitation received during the preceding year (July through June) and a function of time that described how flowering decreased after the initial post-fire bloom. We used precipitation records from a weather station in Redlands, California, approximately 20 km southeast of the study site, due to the incomplete nature of precipitation records closer to the study site (NOAA 2020). One month of precipitation data was missing from the Redlands record, so it was estimated from the rainfall patterns observed at

nearby stations. To validate the model, flowering data was taken periodically after the study (in 2014, 2016, and 2019) and compared to model predictions. Regression analyses were performed with JMP statistical software (JMP Pro 15.0.0, SAS Institute Inc., Cary, NC).

Interannual Variation in Leaf Size and Herbivory

During the early-season surveys we mapped and marked all newly encountered *Calochortus* plants in the plots. Those too small to flower were identified to genus only, because *Calochortus splendens* also occurred in the area (Williams et al. 2006) and was difficult to distinguish from *C. plummerae* prior to flowering. We measured the strap leaves of all *Calochortus* plants (length and width) and noted any herbivore damage to the leaves. Herbivory that left less than 3 cm of leaf length was arbitrarily scored as “severe” herbivory for the purposes of quantifying year-to-year variation in herbivore damage. Apart from 2009, when we missed the early-season survey, these surveys were conducted from 2007 to 2013.

Effect of Simulated Herbivory on Flowering

To determine the effect of herbivory on flowering of *C. plummerae*, we simulated herbivory by clipping leaves. On 17 February 2013, 24 plants with large strap leaves (>1.0 cm in width, median length ~ 46 cm) were located along the ridges of Badger Hill. We caged half of the plants with hardware cloth (16 mm mesh) to prevent herbivory, and we clipped the strap leaves of the other half to simulate herbivory. Leaves were clipped to a length of 3 cm. Plants were assigned to treatments in a stratified-random manner based on leaf size: plants were matched by leaf width then assigned at random to either the control or clipping treatment. All plants were mapped and marked with a large nail inserted 3 cm east of the plant. Plants were censused on May 10 to determine whether they had flowered or not. The effect that clipping had on the probability of flowering was tested with a Fisher’s exact test.

RESULTS

Flowering, Survival and Dormancy

As expected, the number of *C. plummerae* plants flowering was highest in the first year after fire (Fig. 1A). Year to year variability was high. Flowering completely failed in the driest year (the 2006–2007 season) but reached a level equivalent to 27% of the post-fire bloom eight years after fire (in 2011; Fig. 1A). Plants that bloomed in 2004, right after the fire, constituted a smaller and smaller fraction of the blooming population as the years passed, but one survivor still bloomed in 2013 (Fig. 1A).

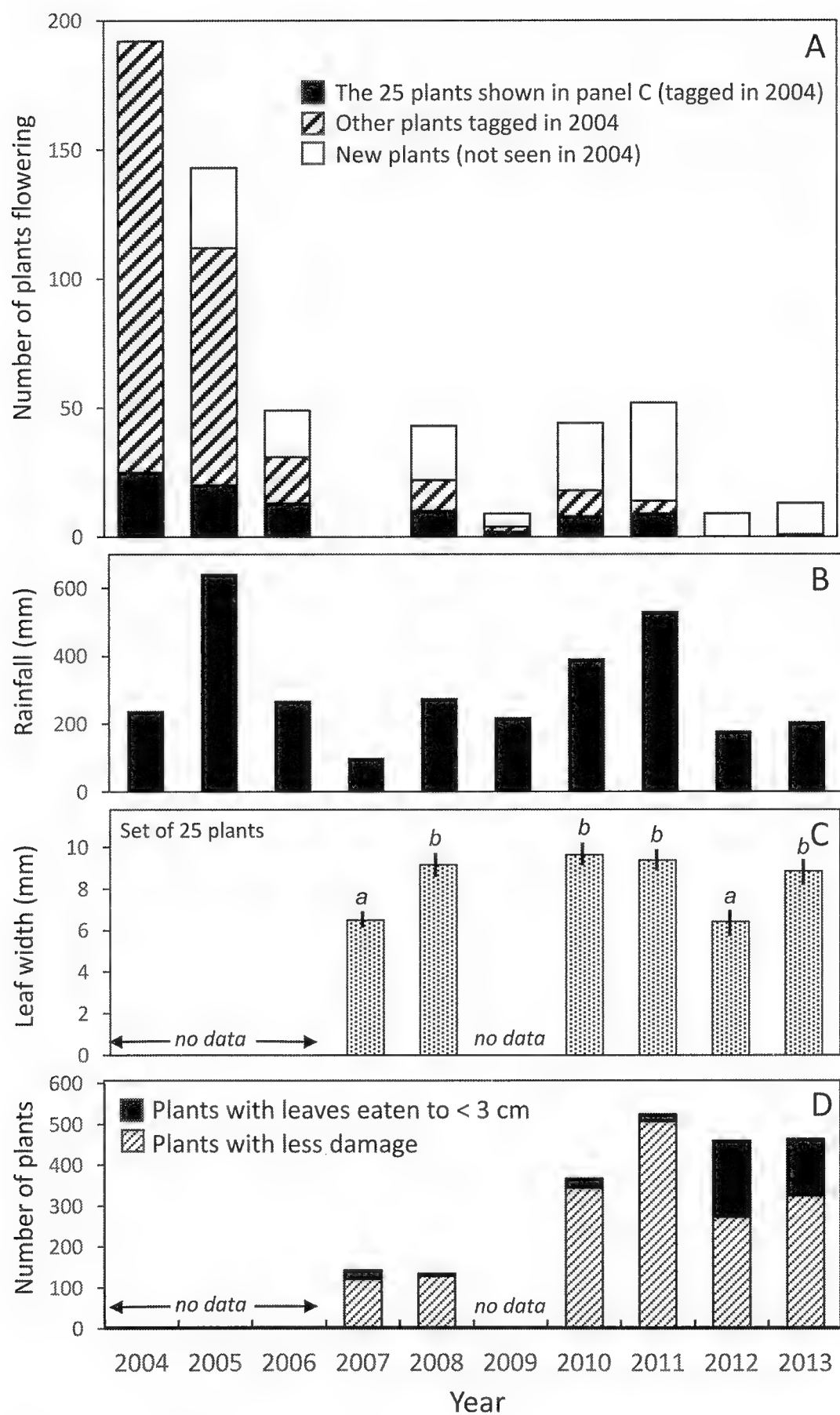


FIG. 1. Year-to-year variation in plant performance (flowering and leaf width) and potential controlling factors (rainfall and herbivory). A. Total number of *C. plummerae* plants flowering in plots. Stacked bars show flowering plants that were originally marked in 2004, separating the set of 25 plants included in the leaf-width analysis (black bars) from others (hatched bars), and flowering plants that were new, or not encountered in 2004 (white bars). B. Rainfall from July of the previous year through June at Redlands, CA. C. Leaf width (mean \pm SE) of 25 individuals for which measurements were available from every census. Bars labeled with the same letter were not significantly different from each other according to multiple pair-wise t-tests with a Bonferroni correction. D. Number of plants of *Calochortus* spp. that experienced severe herbivory (leaving < 3 cm of leaf length; solid bars) and those that experienced less severe or no herbivory each year.

Thirty percent of the originally marked plants were observed in 2013, having survived at least 10 years. The fraction of the originally marked population that produced a leaf during the six years with early-season surveys (i.e., were neither dead nor dormant) ranged from 25% to 30% (25% in 2007, 28% in 2008, 32% in 2010, 31% in 2011, and 30% in both 2012 and 2013). Approximately 60% of the

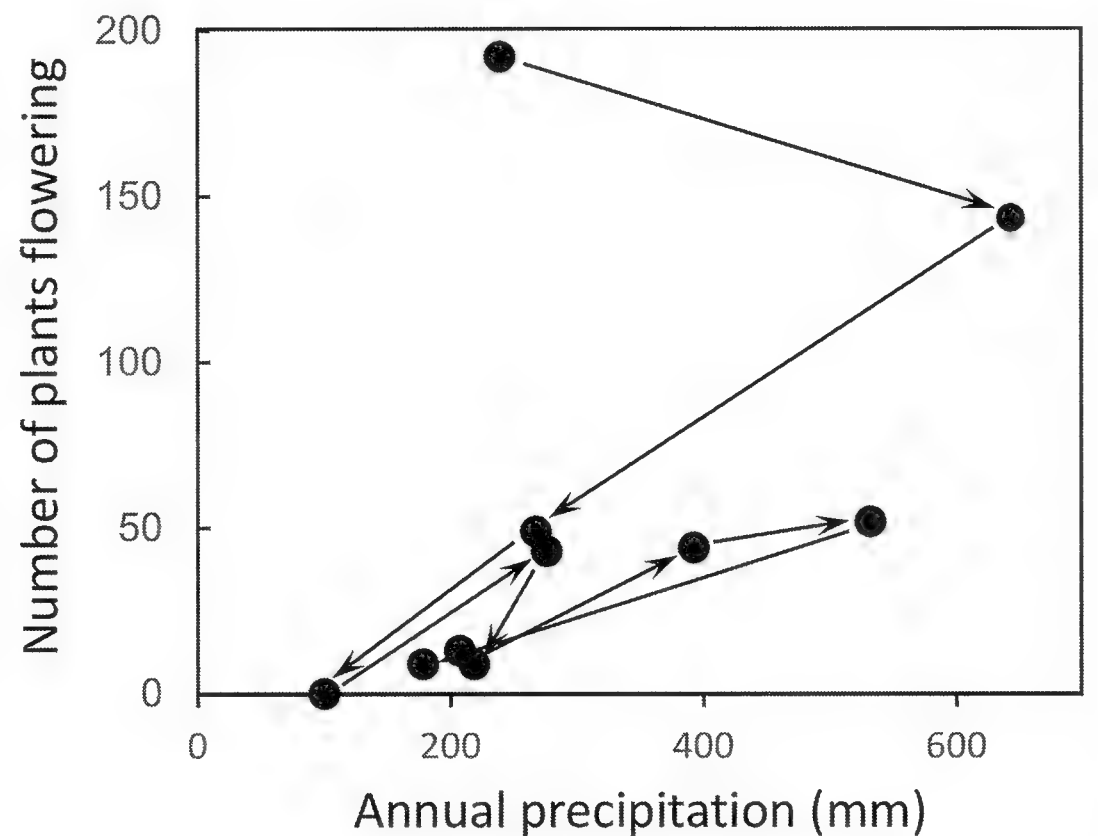


FIG. 2. Relationship between annual rainfall (July–June) and number of *C. plummerae* plants flowering in plots at the end of the season (late May to early June). Arrows indicate the trajectory over time, from the number of plants flowering in 2004 (top) to the number flowering in 2013 (bottom).

originally marked plants were not observed at all during the last seven years of the study and were, therefore, presumed dead. The remaining 10% were not observed during the last year of the study, but we could not determine whether they were dead or merely dormant.

Of those plants that were found in the last year of the study, only 58% were observed above ground every year during the six years with early-season surveys. The rest were not observed during some years, reappearing after a one year “absence” (28%) or an “absence” of two or more years (14%). These “absent” survivors were either dormant, or their leaves were eaten so early and so thoroughly that they could not be detected.

Flowering and Rainfall

The statistical effect of rainfall on flowering only became significant when a second factor was included in the model to account for the stimulatory effect of fire. Annual rainfall varied widely over the course of the study, ranging from 100 mm in the 2006–2007 season to 643 mm in 2004–2005 (Fig. 1B). The number of plants that flowered in a year was not well predicted by annual rainfall alone ($R^2 = 0.22$, $P = 0.17$). However, examination of the trajectory of flowering over the years suggested that flowering did increase with rainfall, but that the effect decreased over time (Fig. 2). A multiple-regression model that incorporated both rainfall and an asymptotically decreasing effect of time since fire yielded a good estimation of the number of *C. plummerae* plants flowering in our plots over the 10 years of this study ($R^2 = 0.98$, adjusted $R^2 = 0.97$, $P < 0.001$). It also predicted increased flowering in a subsequent wet year (28 plants in 2019) fairly closely (Fig. 3). The equation obtained was

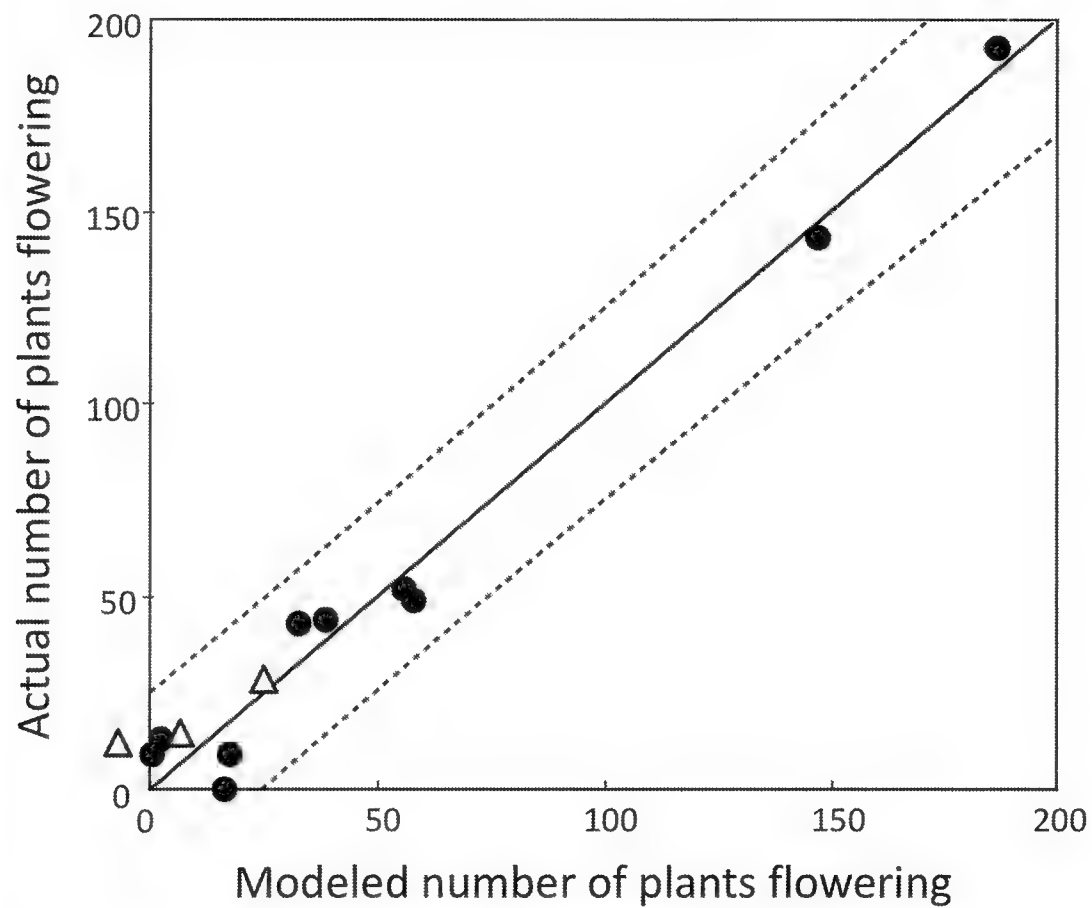


FIG. 3. Number of plants flowering vs. model estimate based on precipitation and time since fire. Closed circles represent data used to form the model, and the dotted lines represent the 95% prediction interval for those data. Triangles represent data taken later, in 2014, 2016, and 2019. Unrealistic predictions of negative numbers of plants flowering in dry years was the result of the model being a simple regression model with a negative intercept, rather than a biological or mechanistic model.

$$F = 0.148R + \frac{199}{T} - 48$$

where F was the number of *C. plummerae* plants flowering in plots, R was the annual rainfall the preceding year (mm), and T was time since fire in years, with year one being the spring following fire. Both rainfall (t-test, $t = 6.97$, $P < 0.001$) and the inverse of time since fire (t-test, $t = 15.37$, $P < 0.001$) contributed significantly to the predictive capacity of the model.

Interannual Variation in Leaf Size and Herbivory

The size of the leaf produced by a plant varied from year to year (Fig. 1C). Since leaf length was often affected by herbivory, we took leaf width as a better measure of the size of leaf that was produced by a plant. Furthermore, to focus on year-to-year variation in the leaf widths of the same individuals, we only used data from *C. plummerae* plants for which we had adequate leaf-width measurements from every early-season census (i.e., not grazed below the widest part of the leaf). Only 25 plants met this criterion. The leaf widths of these plants varied significantly among years (repeated measures ANOVA, $F_{(5,120)} = 15.95$, $P < 0.001$). Average leaf width of these individuals was not well predicted by a simple regression model based on annual rainfall ($R^2 = 0.59$, $P = 0.07$). However, plants produced significantly narrower leaves during the two driest years (post-hoc pairwise t-tests on square-root-transformed data to improve normality with a Bonferroni-adjusted alpha level of 0.0033 (0.05/15); Fig 1C).

Leaf damage by herbivores also varied substantially over the 6 yr it was measured. Among all *Calochortus* plants in our plots, including those identified only to genus, severe herbivory (scored as herbivory that left 3 cm or less of leaf length) ranged from 3% in 2011 to 41% the following year (Fig. 1D). Although insect herbivory (small bites, often along the edges of a leaf) was apparent throughout the study, damage scored as severe herbivory was usually the result of straight cuts and likely the work of small mammals. Both rabbit scat and rodent activity (soil disturbance) were observed in the plots.

Effect of Simulated Herbivory on Flowering

Simulated herbivory (leaf clipping) in February 2013 had a dramatic effect on flowering later the same spring. Four experimental plants could not be found in May, including three plants that had had their leaves clipped and one that had been caged. Of the 11 caged plants that were found, ten produced flowering stalks. Of the nine clipped plants that were found, none produced flowering stalks. This difference was highly significant (Fisher's exact test, $P < 0.0001$).

DISCUSSION

Flowering of *Calochortus plummerae* was not completely restricted to the first year or two immediately after fire, but flowering declined substantially after the initial post-fire bloom. After the second year post-fire, the density of flowering plants did not exceed 27% of that observed immediately after fire. The mechanisms responsible for both the post-fire bloom and the decrease in flowering over subsequent years were not addressed in this study but most likely include changes in plant competition. Competition was very low the first year after fire, with plots supporting only sparse growth of annual plants and very small resprouts of chaparral shrubs (primarily *Adenostoma fasciculatum* Hook. & Arn.). Although chaparral recovery was not formally measured, it followed a typical trajectory. *Acmispon glaber* (Vogel) Brouillet, a rapidly growing subshrub, dominated some areas with cover peaking the third year after fire (2006). The crowns of *Adenostoma fasciculatum* continued to increase in size throughout the study, and their heights surpassed those of the faster-growing *Salvia* species (*Salvia mellifera* Greene and *Salvia apiana* Jeps.) by the fifth or sixth year of the study. Although changing competition from shrubs and subshrubs may have played a major role in the asymptotically decreasing trajectory of flowering in *C. plummerae* over the years, we cannot rule out other factors. "Time since fire" subsumed all changing factors related to post-fire recovery, allowing us to test effects of factors unrelated to fire cycles, such as variation in rainfall.

Flowering of *C. plummerae* was well predicted by annual rainfall once the overall effect of "time since fire" was factored out. This was true both at a

population level and when individual plants were followed (Fig. 1A). The set of 25 plants that consistently had measurable leaves during early-season surveys produced narrower leaves and were less likely to flower during dry years than during wetter years. Increased plant dormancy may have contributed to low flowering during dry years, but data supporting increased dormancy during dry years was weak. The fraction of the originally marked population that was active (produced a leaf) in years with early-season censuses only ranged from 25% to 32%. Even during the driest year of the study (2007), a quarter of the originally marked plants produced leaves. Plant dormancy, therefore, could not account for the complete lack of flowering that year.

Over the 10 yr of the study, there was substantial turnover in the population that flowered. A large fraction of the originally marked population (60%) was not observed at all during the last 7 yr of the study. These plants were either very deeply dormant or dead. We assumed, however, that they were dead. Studies of bulb dormancy in other *Calochortus* species have found either little evidence of dormancy (e.g., Fiedler 1987; Fredricks 1992) or a pattern similar to the pattern we found for *C. plummerae*, with the largest fraction of apparently dormant plants producing leaves after a dormant period of only one year, a smaller fraction producing leaves after a dormant period of 2 yr, and an extremely small fraction producing leaves after a dormant period of three or more years (Miller et al. 2004). The fact that more than a quarter of our originally marked plants put up leaves every year or skipped periods of only one year make it unlikely (although possible) that other individuals experienced such deep dormancy that they failed to produce leaves for 7 yr in a row. As years went by, new individuals began to flower. After 7 yr, the originally marked plants accounted for less than half of the flowering population.

The fact that leaf area removal (simulated herbivory) eliminated flowering later the same year suggests that flowering in this species is largely dependent on current-year carbon gain. There is good evidence that carbon gain in prior years and the size of the underground storage organ influence flowering of various other geophytes (e.g., Dafni et al. 1981; Tyler and Borchert 2003; Schlising and Chamberlain 2006; Borchert and Tyler 2009). Moreover, the flowering of some geophytes appears to exhaust their reserves to the extent that they grow more slowly, remain dormant, or fail to flower the following year (Eggert 1992, Tyler and Borchert 2003; Lesica and Crone 2007). Tyler and Borchert (2003) suggested that a decline in flowering of some geophytes in the years following the first post-fire year may be due, in part, to the depletion of storage reserves caused by the post-fire flowering, itself. We did not see patterns in our flowering data to suggest that the act of flowering exhausted reserves of *C. plummerae* plants to such an extent that they failed to flower the next year. In fact, a few individuals

flowered 3 yr in a row between the fifth and tenth years after fire. The dynamics of carbon gain and depletion in prior years may play a role in the flowering of *C. plummerae*, but our data suggest that current-year carbon gain is a critical determinant of flowering in this species. The pattern of increased flowering in wet years and in the period after fire (with consequently higher levels of soil nutrients and lower competition for light and water) is consistent with effects of increased resources on carbon gain and with the observation that flowering is dependent on current-year carbon gain.

Although resources released by fire may largely account for prolific flowering after fire, large fires can also reduce herbivory (Christensen and Muller 1975; Barro and Conard 1991). Patterns of herbivore damage on *C. plummerae* at our site suggested that mammalian herbivores were responsible for most instances of severe herbivory. This observation is consistent with those of Fiedler (1987) who noted that the most damaging herbivory on four *Calochortus* species that she studied along the central California coast was caused by small mammals (several rabbit species and pocket gophers) and by mule deer. While burrowing animals may survive fires, large fires have been reported to cause heavy mortality among herbivores such as brush rabbits (replaced by desert cottontails at our site) and California ground squirrels (Chew et al. 1959; Quinn 1990; van Mantgem et al. 2015). Mule deer may die in fires (Chew et al. 1959) but often flee fire and recolonize the area in the years after fire (Quinn 1990; van Mantgem et al. 2015). Therefore, herbivore damage to *C. plummerae* plants at our site may have lowest in the years immediately following fire, before we began monitoring it.

One apparent paradox in our findings was that herbivory had a strong effect on flowering, and severe herbivory varied among years, but we did not need to include herbivory in the model to obtain a good prediction of flowering. Since herbivore populations have been shown to respond to both fire and rainfall, usually in complex and species-specific ways (e.g., Christensen and Muller 1975; Barro and Conard 1991; Holmgren et al. 2001; van Mantgem et al. 2015) it is possible that impacts of herbivory on flowering were subsumed under either the “time since fire” effect or the “rainfall” effect.

Regardless of the causal mechanisms, we found interannual patterns of flowering in *Calochortus plummerae* that were consistent both with fire-promotion of flowering and with flowering that was controlled increasingly by direct or indirect effects of precipitation during the fire-free interval. Flowering was eliminated during severe drought, and this flowering failure occurred despite the continued presence of non-dormant plants on the site. Although high rainfall during the fire-free interval appeared to stimulate flowering of *C. plummerae*, the density of flowering plants in this recovering

shrubland vegetation was rarely more than a quarter of that found during the initial post-fire bloom.

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GROWTH RESPONSES OF *LASTHENIA GRACILIS* TO SIMULATED DROUGHT

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ABSTRACT

Climate change impacts the severity and frequency of droughts in California. As a result, native plants will likely face changes in soil moisture as temperature and weather patterns shift. *Lasthenia gracilis* (DC.) Greene, commonly known as Needle Goldfields, is a native California wildflower distributed across broad climate gradients from northern to southern California. Therefore, this species is ideal for testing how water availability affects populations from contrasting locations across its range. In a greenhouse experiment, we measured the effects of differing water treatments on the growth and reproductive fitness of this species to explore if there is variation in response between northern and southern populations. We also measured days to germination, germination rate, and days to flowering to further compare responses among the geographically distinct populations. Individuals from most populations exhibited faster growth rates when exposed to more water. The response of reproductive output (number of inflorescences) to the treatments was reduced in populations historically exposed to drought conditions, suggesting that the southern populations have lower population plasticity for this trait. Further, individuals in five out of six populations produced inflorescences later and flowered for longer when exposed to the highest watering treatment, revealing that phenology is significantly impacted by differing water treatments. Studying the effects of limited water availability on growth and reproductive ability in plant populations across a species' geographic range can provide more complete insight into how climate change may impact a species. This study indicates the presence of relative population plasticity in response to drought, which may be important to consider during restoration planning.

Key Words: climate change, drought, greenhouse experiment, growth, phenology, population plasticity, reproduction, restoration.

Anthropogenic climate change is causing an increase in global average temperatures, sea level rise, changes in precipitation patterns, and disturbances to species interactions (Karl et al. 2009; Kopp et al. 2014; Kharouba et al. 2018; Renner and Zohner 2018; Fawzy et al. 2020; Olliff-Yang et al. 2020). California is experiencing an increase in the frequency, magnitude, and length of droughts with “chronic, long-term hydrological drought” looming at the end of this century (Mann and Gleick 2015; Wuebbles et al. 2017). Furthermore, global temperature increases of 1°C have already been recorded, with business-as-usual scenarios predicting a global average increase of up to 5°C by 2100 (Wuebbles et al. 2017). Both extended drought conditions and increasing temperatures have been correlated with earlier flowering (Cui et al. 2017; König et al. 2017; Papper et al. 2021; Pearson et al. 2021), a potentially detrimental phenomenon to the community composition and ecosystem-level resilience of flowering grassland species (Suttle 2007; Crimmins 2009).

Water stress, especially the prolonged periods without precipitation associated with drought, can disrupt the phenology of plants (Suttle 2007). Phenology is the timing of biological events in a life cycle such as flowering, breeding, and hibernation (Lieth 1974). Variability in phenology has been found to be higher in early-flowering plant families (Mazer et al. 2012), grasses (Munson and Long 2017), and early flowering species (Wainwright et al.

2012). Specifically in mid- to high-latitudes, warming temperatures disproportionately affect the phenology of early-active flowering plants due to higher temperature variability in spring months (Menzel et al. 2006). Changes in phenology can have serious repercussions for both individual plant fitness and entire ecosystems (Cui et al. 2017; Mazer et al. 2012). At an extreme, alterations to the phenology and reproductive ability of species can lead to phenological mismatches — asynchrony with pollinators or a loss of temporal overlap between mutualistic species (Rafferty et al. 2015; Renner and Zohner 2018, Olliff-Yang et al. 2020).

Conversely, some species may benefit from phenological shifts in response to changing environmental factors. For example, species with minimal photoperiod and chilling requirements (time exposed to low temperature required to break dormancy) may actually increase in abundance and distribution due to earlier budbreak (producing green leaves after dormancy) (Polgar and Primack 2011; Koenig et al. 2021). In addition, species that exhibit less phenological shift in response to climate changes have decreased greatly in abundance, a trend observed in over 400 flowering plant species (Willis et al. 2008). Hence, shifting in flowering time may have costs, such as potential mismatch or changed species interactions, and also benefits, such as the ability to track optimal temperatures for growth.

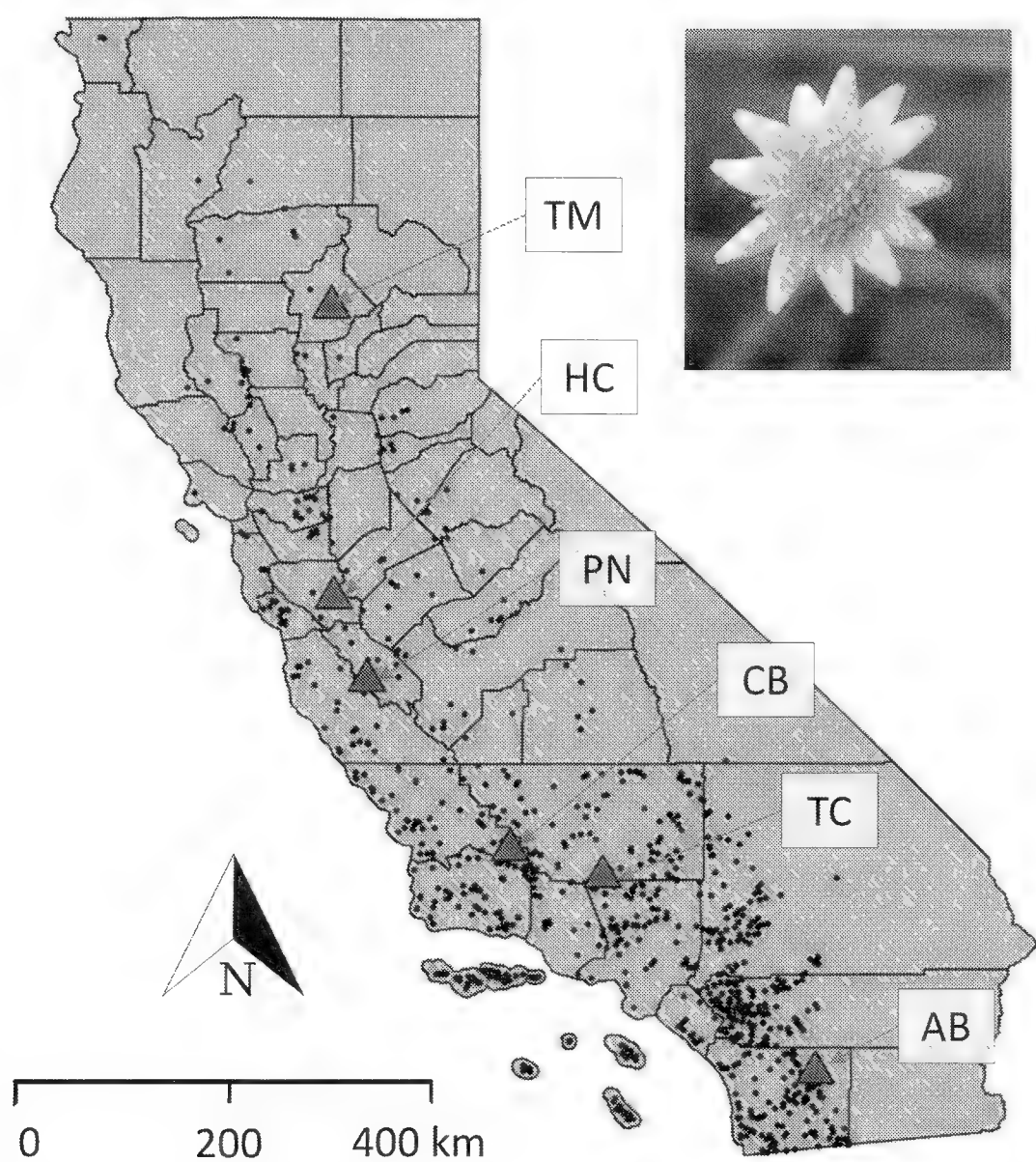


FIG. 1. All California Consortium of Herbaria (CCH) records of *Lasthenia gracilis*. Black points are herbarium specimen collection locations, grey triangles are seed collection locations for populations used in this study. Collection data were cleaned and georeferenced using methods described in Baldwin et al. (2017).

Importantly, there is evidence of adaptive differentiation within a species in response to different amounts of precipitation in the environment (Sultan 1996; Rajakaruna et al. 2003). For example, Sultan (1996) found that the offspring of *Polygonum persicaria* L. exposed to various environmental stressors, including low soil moisture, demonstrate wide plasticity in their growth responses due to higher provisioning of mass to seedlings, earlier germination time, and other individual parental compensations. Similarly, Rajakaruna et al. (2003) recorded plasticity in *Lasthenia californica* (DC.) Greene *sensu* Ornduff (1966, 1993) when exposed to drought conditions and found them to be adaptively differentiated by population for reproductive responses but not for growth responses. This phenotypic plasticity, at the individual and population level, could provide species with a wide geographic range with greater drought tolerance and increased resistance to competition caused by phenology shifts (Nicotra and Davidson 2010; Wainright et al. 2012; Pearse et al. 2020).

The purpose of this study is to determine intraspecific variation to drought stress in a California native herbaceous annual. We are studying this response using *Lasthenia gracilis* (DC.) Greene (Asteraceae), as a model organism. We chose this species because it is a flowering annual with a large geographic range, genetically-distinct populations (Rajakaruna 2003), and relatively short growth cycle (Rajakaruna and Bohm 1999; Calflora 2018). Flow-

ering time in *Lasthenia* species is responsive to water availability (Emery 2009), and there is genetic variation within a population of vernal pool species for responses to water depth and season length (Emery and Ackerly 2014). *Lasthenia* is also used in California restoration projects, making it relevant to future management decision applications (Point Blue Conservation Science 2019). In this study, we test for differences in population-level plasticity of the growth and phenology of an early-flowering native California annual exposed to differing amounts of water. These differences could be adaptive assuming the populations are adapted to their geographic areas of origin. We hope to answer the following questions: 1) Does *L. gracilis* exhibit plasticity in growth rate, inflorescence number, and flowering time in response to different levels of water, and 2) do plastic responses to water treatments differ based on source location, indicating adaptive differentiation?

METHODS

Study Species

We investigated the effects of different watering treatments on the growth of individuals from six populations of *L. gracilis*, a native California wildflower with a range of habitats from northern California to northwestern Baja California (Calflora 2018). *Lasthenia* is a genus comprised of 18 known species and subspecies occupying a diverse set of habitats (Chan and Ornduff 2000). These include coastal bluffs, open grasslands, oak woodlands, alkali flats, chaparral, pastures, roadsides, desert habitats, and serpentine outcrops (Rajakaruna and Bohm 1999; Rajakaruna 2003). We chose *L. gracilis* as our model organism because it is a flowering annual with a large geographic range (Fig. 1) with evidence indicating genetically-distinct populations (Rajakaruna 2003, Montalvo et al. 2017). This makes it favorable for investigating adaptive differentiation in the context of drought conditions (Rajakaruna and Bohm 1999; Rajakaruna et al. 2003). This is an obligately outcrossing species, requiring cross-pollination to set seed (Ornduff, 1966). *Lasthenia gracilis* has a typical bloom period from February to June (Calflora 2018), relatively fast experimental germination (1–2 wk) and short growth cycles (2 mo). Due to hypothesized adaptive differentiation, the suitable temperature range, elevation, amount of precipitation, and morphological characteristics are extremely broad and varied. For instance, the wet season within *L. gracilis* species range is anywhere from 0 to 8 mo with average temperatures ranging from 21°F to 64°F (Calflora 2018).

Study Population Selection

We chose six populations based on latitude, climate moisture deficit (CMD), and seed availability. From north to south the populations are Table

Mountain (TM), Henry Coe (HC), Pinnacles (PN), Carrizo Plain (CB), Tejon Mojave (TC), and Anza Borrego (AB) (Appendix 1). Maternal effects were removed from 14 maternal lines by growing one generation in the greenhouse under uniform conditions, and by cross-pollinating individuals by hand. Seeds were collected and pooled from inflorescences that had been cross-pollinated with another individual within the same population and separated to prevent accidental pollen transfer between populations. We pooled 10 seeds each from five maternal lines from each population to germinate for our greenhouse study. We selected populations with at least 13 successfully-germinated seeds during an initial germination trial and excluded populations that did not germinate sufficiently to include enough replicates. Our populations represented a range from arid, southern habitats to wetter, northern habitats (Appendix 1, Fig. 1).

Greenhouse Experiment

To determine the relationship between population and growth response to differing water treatments, we conducted a greenhouse experiment in 1.5" diameter planting cones of Sunshine Growth 4 Aggregate Mix (Sun Gro Horticulture, McClellan Park, California) at the Oxford Tract greenhouses at the University of California, Berkeley. We controlled temperature, light, and pest exposure. The greenhouse was maintained at a temperature from 64–77°F with a photoperiod of 12 hr of light from overhead high intensity discharge (HID) lights to simulate average temperature of the growing season across California. Neither pesticides nor fertilizer was used during this experiment.

Experimental Methods

Germination. To test the effects of three watering treatments on growth and reproduction, we exposed four individuals from each of the six populations to one of three watering treatments ($n = 12$ plants per population). As a buffer for replicate loss, we added one extra seed per population, totaling planting 13 individuals from each of the six populations ($n = 78$ plants total). To do this, we germinated 50 seeds from ten populations (including the six populations listed above) to compensate for variable germination rates between populations. For each population, we pooled ten viable seeds (determined based on color [dark brown to black] and fill [opaque]) from five randomly chosen mother lines. Next, we placed the seeds in Petri dishes with filter paper pre-moistened with 1–5 mL of deionized water. We then placed the dishes in a refrigerator at 2°C until root tips emerged to mimic the cold, dark germination conditions characteristic of winter in California. We selected six populations of the original ten (four not shown) based on successful germination and to optimize the

diversity in terms of latitude between populations (Appendices 1, 2).

Planting. We transferred pre-germinated seeds into sterilized cones containing water-saturated Sunshine Growth 4 Aggregate Mix. We prepared containers by cleaning them with a 10% bleach solution and rinsing them with tap water. To prevent soil loss, we placed a jumbo cotton ball in the bottom of each cone and then filled them with Sunshine Growth 4 Aggregate Mix. To facilitate precise seedling transfer, we fully saturated soil by adding tap water, allowing the soil to settle, refilling each cone with approximately one cup of additional soil, and bringing the dry soil to saturation. We also employed bottom watering to keep the soil saturated for the first 17 d of growth, changing the water once a week to prevent algae growth. To prevent breakage due to pinching, we used tweezers to lift one seed at a time into previously-created indents (~0.5 cm deep) in each cone. To ensure contact between the root hairs and the soil, we gently pushed soil around the seed and moistened the area with 1–5 mL of water. We moved any large pieces of perlite away from the seed with tweezers to prevent desiccation. To randomize placement, we organized the 72 cones (4 individuals exposed to each treatment \times 3 treatments \times 6 populations) according to a random number generator and labeled them with the population and seed number.

Water treatments. We established three watering treatments that ranged from saturated soil to dry conditions and exposed four individuals from each population to each treatment. Our watering treatments were as follows: Low, 10 mL of tap water once per week; Medium, 10 mL of tap water twice per week; High, 25 mL of tap water twice per week, maintaining soils at saturation. We determined the prescribed watering volumes by observing the moisture level at the surface of several cones (to attain saturation throughout the cone for the highest watering treatment, surface dryness and root moisture for the medium watering treatment, and total dryness between lowest watering treatments) over a month-long pilot study we initiated 2 wk before the greenhouse experiment. Plant water potential measurements were not conducted, however, a similar frequency and watering amounts (scaled to pot size) were used in a previous study that investigated the response to water stress in *Lasthenia* (Rajakaruna et al. 2003).

At least 13 seeds from each population germinated in the span of 8 d. After germination, seedlings were transferred from the petri dishes to the planting cones. We kept the planted seedlings moist by bottom-watering and spraying the soil surface with approximately 5 mL of water once a day. We stopped moistening the top of the soil 3 d before the watering treatment began and removed the planting cones from bottom-watering tray on the day the watering treatments began (17 d after planting). We continued

TABLE 1. MEANS OF GROWTH RESPONSES BY POPULATION AND WATER TREATMENT. Watering treatments are shown in columns (labeled as “Low”, “Med” and “High”) and rows indicate different populations.

Population	Maximum height (cm)			Growth rate over first 51 days (cm/d)			Length of longest leaf at first flower (cm)		
	Low	Med	High	Low	Med	High	Low	Med	High
TM	8.88	11.33	16.15	0.164	0.209	0.308	2.90	2.78	4.18
HC	10.53	11.23	12.65	0.203	0.216	0.250	3.85	4.58	4.78
PN	10.00	9.33	12.75	0.190	0.178	0.247	3.23	3.33	3.20
CB	10.20	10.18	9.98	0.195	0.197	0.192	2.68	3.13	2.78
TC	10.05	7.55	10.30	0.189	0.138	0.193	3.45	2.48	2.63
AB	10.08	10.68	14.18	0.192	0.204	0.278	3.20	3.38	5.23

watering according to this scheme until most individuals reached senescence and stopped producing flowers. The greenhouse experiment was initiated on October 26, 2018 and ran for 101 d. Since four individuals were exposed to each water treatment from each population, our experimental design was balanced.

Data Collection

To quantify the effects of differing water treatments, we recorded seedling growth metrics over 101 d. We chose growth rate (cm/d), inflorescence counts, days to flowering (days), peak flowering time (days since planting), and length of longest leaf at first flower (cm) because they represent growth, phenology, and reproductive fitness. We collected data at least two times per week for 12 wk. To find the relationship between differing water treatments and growth, we measured the distance from the top of the soil to the tallest part of the plant (whether it was the apical meristem or the tip of the longest leaf) two times per week using a tape measure. These heights divided by the total number of days during which the plant’s height is positively increasing gave the growth rate for that plant. We also measured the length of the longest leaf on the day that each plant opened its first flower, as a measure of relative leaf differences at the time of flowering.

To find the relationship between differing water treatments and reproductive fitness and phenology, we collected inflorescence counts twice a week. Characteristic of Asteraceae, *L. gracilis* produces a cyme-like head comprised of both disk and ray florets (Keil 2017). To determine days to flowering, we recorded the date that each plant exhibited its first “open inflorescence”, defined as having at least one open ray flower in an inflorescence. We then used this date to determine the number of days until flowering from the date of planting. We counted the number of open inflorescences on each plant every day until most populations, with the exception of PN, TM, and AB, had reached senescence, defined as the point when all inflorescences were counted dead. Plants in PN and AB exposed to the high water treatment (and plants from TM in other treatments) generally continued to flower, although the inflorescences at the end of the study were small and had very few

open ray and disk flowers. These daily measurements gave the inflorescence counts and were used to determine the peak flowering time.

Data Analysis

To determine the relationship of the growth and reproduction response variables with both population and water treatment, we employed various methods of statistical analyses in R (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). For each of the seven dependent variables (growth rate, maximum number of inflorescences, length of longest leaf at first flower, flowering start date, flowering peak date, flowering end date, and flowering duration) we performed a two-way analysis of variance (ANOVA), with population and water treatment as fixed predictor variables. We also tested the interaction between population and water treatment on the response variables. Initial height was included as a covariate in the ANOVA models to control for any differences in seedling size at planting. To check the normality of our data we used Shapiro-Wilks tests and examined the histograms of the residuals.

To visually compare the variation in growth responses to drought conditions among populations, we created ordered boxplots, arranged from south to north by latitude (see Appendix 1 for latitudes and longitudes). We plotted the average of the growth rate on the Y axis for individuals from each population grouped by water treatment on the X axis to look for clusters of like responses by population and trends. We repeated this for the maximum number of inflorescences and length of longest leaf at first flower. To visually interpret the phenological responses, we created two horizontal boxplots of the start, peak, and end flowering dates grouped both by population and watering treatment.

RESULTS

Maximum Height

Both population and watering treatments significantly impacted growth variables (height, growth rate for the first 51 d, length of longest leaf at first

TABLE 2. RESULTS OF TWO-WAY ANOVAS TESTING THREE DEPENDENT VARIABLES ~ POPULATION + WATER TREATMENT + POPULATION × TREATMENT + INITIAL HEIGHT. * denotes significant effect ($\alpha = 0.05$).

Dependent variable	Source	Type III sum of squares	df	Mean square	F	P-value
Maximum height	Population	87.98	5	17.60	3.392	0.010*
	Water treatment	93.87	2	46.93	9.047	4.17e−04*
	Population × Treatment	67.92	10	6.79	1.309	0.250
	Initial height	71.06	1	71.06	13.698	5.12e−4*
Growth rate over the first 51 d	Population	0.03906	5	0.007812	3.374	0.010*
	Water treatment	0.03798	2	0.018990	8.202	7.88e−04*
	Population × Treatment	0.03231	10	0.003231	1.395	0.208
	Initial height	0.01986	1	0.019857	8.577	0.005*
Maximum number of flowers	Population	993.1	5	198.61	9.430	1.79e−06*
	Water treatment	332.5	2	166.26	7.894	9.98e−04*
	Population × Treatment	299.5	10	29.95	1.422	0.196
	Initial height	58.2	1	58.18	2.762	0.102

flower), with no significant interaction (Table 2, Fig. 2). This lack of interaction indicates that the effect of water level on growth and flowering did not differ among populations. Maximum height was significantly affected in a two-way ANOVA test by both population ($df = 5$, $F_{5,67} = 3.392$, $P = 0.010$) and water treatment ($df = 2$, $F_{2,70} = 9.047$, $P = 0.0004$) (Table 2). A post-hoc Tukey test indicated that there was a statistically significant difference in maximum heights between the low and high water treatments ($P = 0.006$) and between the medium and high water treatments ($P = 0.009$) but not between the low and medium water treatments ($P = 0.99$). Although the interaction was not significant, plants from TM demonstrated the greatest percent difference in average height and growth rate between the lowest and highest watering treatments (Fig. 1). Most populations produced taller plants with increasing amounts of water.

Growth Rate Over First 51 Days

Water treatment ($df = 2$, $F_{2,70} = 8.202$, $P = 0.0008$) and population ($df = 5$, $F_{5,67} = 3.374$, $P = 0.010$) significantly impacted the growth rates over the first 51 d in a two-way ANOVA test, indicating that the response to increasing amount of water is faster growth (Table 2, Fig. 2). The interaction between population and treatment was not significant. A post-hoc Tukey test indicated that there was a statistically significant difference in growth rates between the low and high water treatments ($P = 0.008$) and between the medium and high water treatments ($P = 0.01$) but not between the low and medium water treatments ($P = 0.99$). The Tukey test also indicated that plants under the highest water treatment showed higher growth rates over all populations.

Flowering

We found a significant positive relationship between amount of water and reproductive fitness.

The maximum number of inflorescences are significantly affected by both population ($df = 5$, $F_{5,67} = 9.430$, $P < 0.0001$) and water treatment ($df = 2$, $F_{2,70} = 7.894$, $P = 0.001$) (Table 2), with no significant interaction in a two-way ANOVA test. Within most populations, individuals treated with the highest watering treatment yielded more inflorescences compared to individuals of the same population exposed to drier conditions. Consequently, plants in all populations except one responded to drought conditions by producing fewer inflorescences (Fig. 2). Historical CMD data from the source locations were correlated with reproduction ($P = 0.003$, correlation = -0.342).

Phenology

We found that the phenology of *L. gracilis* (flowering start date, flowering end date, flowering duration, and peak flowering date) showed significant population and water treatment effects, with no significant interactions (Table 3) in our two-way ANOVA tests. Population showed significant effects on flowering start date ($df = 5$, $F_{5,67} = 3.541$, $P = 0.008$), flowering end date ($df = 5$, $F_{5,67} = 2.911$, $P = 0.021$), and flowering duration ($df = 5$, $F_{5,67} = 4.039$, $P = 0.004$). Treatment showed significant effects on flowering end date ($df = 2$, $F_{2,70} = 8.340$, $P < 0.001$), flowering duration ($df = 2$, $F_{2,70} = 5.773$, $P = 0.005$), and peak flowering date ($df = 2$, $F_{2,70} = 4.108$, $P = 0.022$). Flowers from all populations flowered later and longer when treated with more water (Table 4). On average, the peak flowering date was shifted 7 d later when comparing the highest and lowest treatments for all populations. The end flowering date was shifted 14 d later on average when comparing the highest and lowest treatments for all populations. The duration of flowering was shortened by 9 d on average when comparing the highest and lowest treatments for all populations. There is no discernible pattern of phenological shift when the populations are ordered by latitude (Fig. 3A).

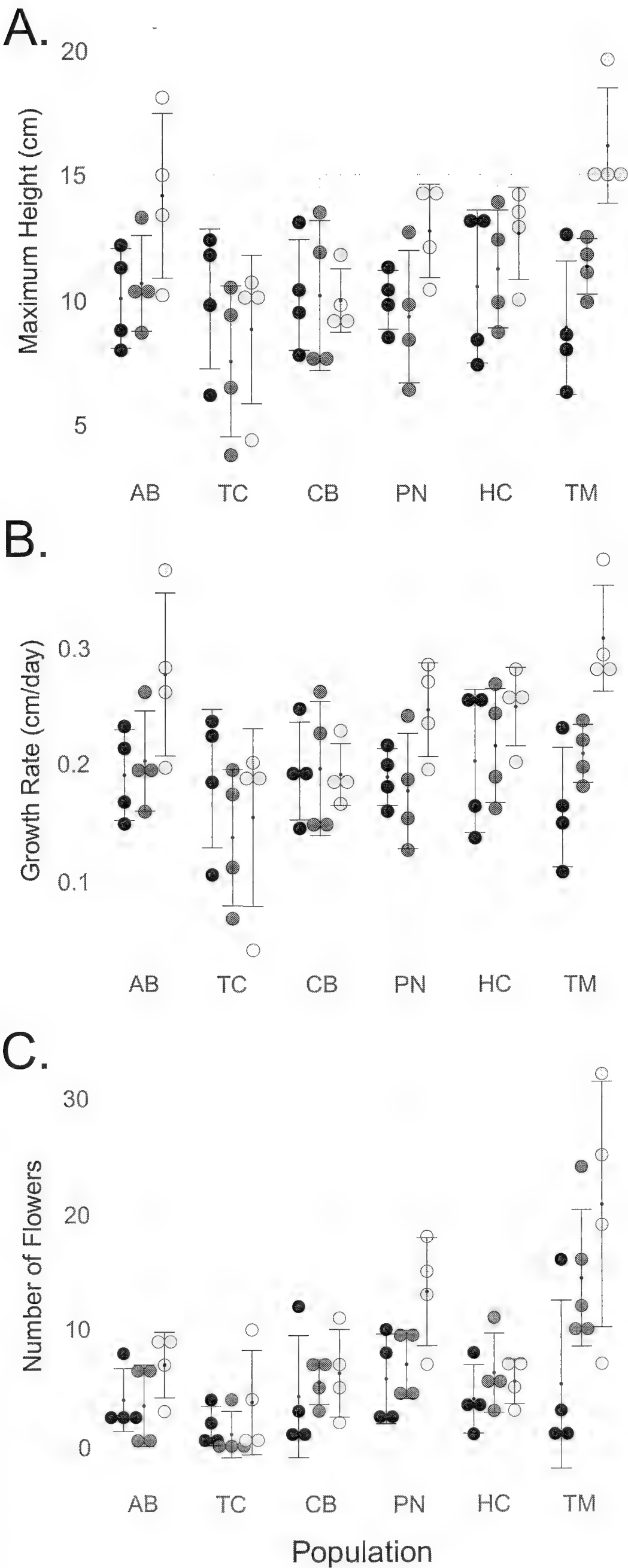


FIG. 2. The average maximum heights (A), growth rates (B), and maximum number of inflorescences (C) ordered by population from south to north and sub-ordered by water treatment. Black indicates low watering treatment; grey indicates medium watering treatment; white indicates high watering treatment. The error bars indicate the standard error of the mean.

DISCUSSION

We found that watering treatments produced significant differences in growth across all populations. In our experiment, plants grew taller and faster with increasing amounts of water when compared to plants of the same population exposed to drought conditions, with the exception of plants from TC and CP. The historical environments of TC and CP populations are on the lower end of average precipitation and highest climate moisture deficits (Appendix 1). Plants from TC and CP could have been allocating more biomass to their root systems, an example of a functional trade-off observed in vascular plants, especially those in desert environments (Michelaki et al. 2019). A future experiment incorporating measurements of pre-drawn water potential and below/aboveground biomass could further investigate this potential drought tolerance strategy. Generally, interaction between population and treatment was not found to be significant for any response variable, so the effect of water level on growth and flowering did not depend on population. Consequently, because *L. gracilis* grows on a wide range of edaphic environments, the range in growth responses to water level fits a hypothesis of high plasticity in this species (Rajakaruna and Bohm 1999; Rajakaruna et al. 2003).

Plants in all populations produced a significantly higher number of inflorescences when treated with more water, with the exception of one population. Historical CMD data from source location was correlated with reproduction in a weak negative linear relationship (correlation not shown). The negative correlation between historical CMD and inflorescence production indicates that populations from wetter environments tend to exhibit higher levels of reproductive output. Plants from AB, TM, and HC under some water treatments continued flowering after the plants from other populations had reached senescence. Inflorescences produced after we had stopped watering were small, low on the plant, and often consisted of just a few open ray flowers poking through a bud. Since we defined inflorescences as having open ray flowers, these small inflorescences with only ray flowers were counted. This has also been seen in other plant species from arid environments — where more water is available seed production increases (Yu et al. 2021), indicating that our flower data is a proxy for reproductive responses to water availability.

This behavior of producing more inflorescences with more water is consistent with resource cost hypotheses which highlight the inherent trade-offs associated with the allocation of water for reproduction by asserting that this can be costly to vegetative growth and ultimately survival (Galen 2005; Tracey and Aarssen 2014). Namely, *L. gracilis* from all populations appear to allocate less water and energy to reproduction when resources are limited. However, smaller plants are able to allocate fewer resources

TABLE 3. THE RESULTS OF TWO-WAY ANOVAS TESTING THE EFFECTS OF POPULATION + WATER TREATMENT + POPULATION x WATER TREATMENT + INITIAL HEIGHT ON FOUR PHENOLOGY VARIABLES. * denotes significant effect ($\alpha = 0.05$).

Dependent variable	Source	Type III sum of squares	df	Mean square	F	P-value
Flowering start date	Population	322.9	5	64.58	3.541	0.008*
	Water treatment	80.2	2	40.10	2.199	0.121
	Populationxtreatment	264.1	10	26.41	1.448	0.186
	Initial height	132.5	1	132.55	7.268	0.009*
Flowering end date	Population	2138	5	427.6	2.911	0.021*
	Water treatment	2450	2	1225.0	8.340	0.001*
	Populationxtreatment	1025	10	102.5	0.698	0.722
	Initial height	87	1	87.1	0.593	0.445
Flowering duration	Population	2900	5	579.9	4.039	0.004*
	Water treatment	1658	2	828.9	5.773	0.005*
	Populationxtreatment	1125	10	112.5	0.783	0.644
	Initial height	435	1	434.5	3.027	0.088
Peak date	Population	653	5	130.61	1.736	0.142
	Water treatment	618	2	309.01	4.108	0.022*
	Populationxtreatment	1053	10	105.35	1.400	0.206
	Initial height	20	1	19.82	0.263	0.610

to all functions overall (Aarssen and Taylor 1992; Younginger et al. 2017). Therefore, to test for tradeoffs, future studies would need to collect data to compare vegetative and reproductive biomass to explore the trade-offs that occur in this species. We ran a post-hoc correlation of the growth rates and maximum flowers and found the correlation to be significant ($P < 0.001$), indicating a trade-off between plant height and reproductive fitness. Biomass data would help to further investigate plasticity in reproductive growth parameters in response to varying levels of water.

The severity of the negative impacts on reproductive output caused by drought conditions is related to geographic population. Specifically, individuals from locations that receive lower amounts of precipitation on average (AB, TC, CP, and PN) show less plasticity in reproductive response when comparing the number of flowers produced by plants grown under the lowest watering treatment with the number produced under the highest watering treatment within each populations (Appendix 1). For example, individuals from AB showed an average difference of three fewer inflorescences produced per plant when comparing those grown under the lowest watering treatment to those grown under the highest watering treatment. Individuals from TM, a site which receives about four times the amount of precipitation in the field compared with AB, showed an average difference of 16 fewer inflorescences per plant when comparing those treated with the lowest watering treatment to those treated with the highest watering treatment. Individuals from these historically drier locations (AB, TC, CP, and PN) are able to reach inflorescence numbers closer to their high watering treatment maximums, even when exposed to drought conditions. Since water use efficiency is a known drought adaptation (Hendry and Day 2005), this trend in differential reproductive output correlated with population suggests that plants from these populations are more drought tolerant.

Plants from all populations flowered significantly earlier and for a shorter amount of time when exposed to drought conditions. These two strategies are both drought adaptations. For example, in desert plants, a lack of water has been shown to stimulate flowering of annuals (Rathcke and Lacey 1985; Shavrukov et al. 2017). Phenotypically, this results in earlier flowering dates when annuals are exposed to drought conditions compared to when they are

TABLE 4. MEANS OF REPRODUCTIVE RESPONSES BY POPULATION AND WATER TREATMENT. Watering treatments are shown in columns (labeled as “Low”, “Med” and “High”) and rows indicate different populations.

Population	Maximum number of inflorescences			Flowering peak date (days since planting)			Flowering duration (days)		
	Low	Med	High	Low	Med	High	Low	Med	High
TM	5	14	21	44.50	50.20	62.25	34.75	45.20	53.25
HC	4	6	6	48.00	48.25	50.50	30.50	44.25	37.75
PN	6	7	13	51.50	55.25	56.00	38.00	41.50	58.25
CB	4	6	6	51.75	57.75	50.75	31.25	33.75	43.00
TC	2	1	4	40.50	44.50	55.25	31.75	19.25	29.75
AB	4	4	7	48.50	40.75	51.75	37.25	38.25	49.75

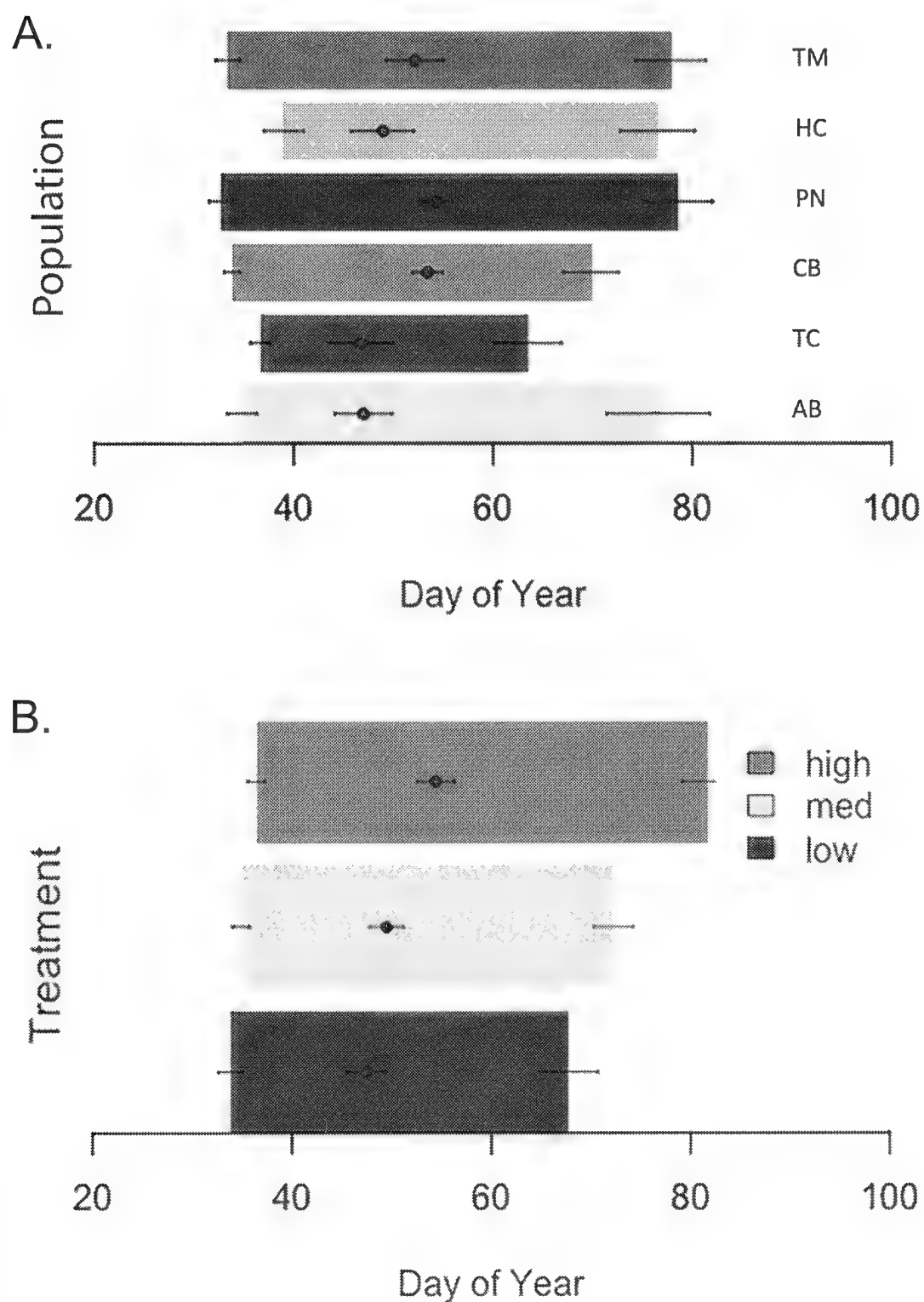


FIG. 3. Average duration of flowering and peak flowering date for each population (A) and for each watering treatment (B). The error bars indicate the standard error of the mean.

given ample water. However, in our study, there is no discernable pattern of stronger phenological shifts correlated with populations that have historically been exposed to less precipitation, as other studies have shown (Pearson et al. 2021). As per previous population-level drought studies, we anticipated that plants from drier locations flower earlier and reach maturity faster, not “betting” on future water supplies (Aronson et al. 1992; Rajakaruna et al. 2003).

There are a few caveats to consider regarding interpretation of our results. First, our growth measurement method was invasive as it required using our hands to pull the delicate plants up to the measuring tape. This resulted in breakage of two plants and one leaf over the course of the greenhouse experiment. Since we had to touch the plants to measure them, our method increased risk but gave valuable growth response data including maximum height and growth rate. We had extra replicates to replace the affected individuals, but we would shift to a less invasive growth measure such as using above and below ground biomass to quantify growth response in future studies. Furthermore, the cones housing the plants were small, so soil got extremely stripped (lost color) and sometimes caked (desiccated

and compacted) by the end of the experiment. Planting in larger pots or implementing micro-tilling (disrupting the soil) could solve this issue.

At any given site, the combination and interaction of countless environmental factors including soil type, temperature, precipitation, local biodiversity, and more have been shown to affect plant growth (Rajakaruna 2003; Rajakaruna et al. 2003; Dierig et al. 2006; Powell et al. 2011). In this project, we investigated the effect of water availability on the growth and reproduction of individuals from six geographically-distinct populations of *L. gracilis* chosen to represent a range of latitudes across California. Since many other factors besides simply the latitude of a site have been shown to affect plant growth, we see potential for a larger drought study to be conducted over various gradients including climate moisture deficit, elevation, temperature, as well as a comparative study of coastal versus inland plant responses.

CONCLUSION

This research on the drought response of a species of native California wildflower has applications in management and ecosystem protection in the face of the changing climate. As California faces more severe and frequent droughts (Mann and Gleick 2015; Wuebbles et al. 2017), information about drought responses of native plants can help predict which species will be impacted the most (Gitlin et al. 2006). Additionally, information about population-level plasticity can help inform population selection in restoration projects. *Lasthenia gracilis* is already included in seed mixtures used for restoration projects and planted along highways by CalTrans because it is good for early cover (Montalvo et al. 2017). *Lasthenia spp.* is also recommended for use in the rehabilitation of disturbed lands because it can tolerate a wide range of environments (Newton and Claassen 2003). Further understanding of the population-based responses to drought can help fine-tune choice of source population for restoration plans based on changing climatic variables.

Additionally, knowledge of environmental preference by population can inform management decisions like the implementation of assisted migration. Assisted migration, or assisted colonization, is a process through which species that are at risk of extinction are introduced to a predicted more suitable environment (Gallagher et al. 2014; Hällfors et al. 2017). For example, survival of translocated White Spruce seedlings declined when seeds were transplanted from wet origins to dry locations due to differences in cold hardiness (Sebastian-Azcona et al. 2019). Some of these population-level limitations can be investigated before launching restoration and assisted migration projects. As California faces a future of more frequent and intense droughts, translocating more drought tolerant lines in areas experiencing more drought could mitigate the possi-

bility of entire loss of a species due to desiccation. Although our results did not indicate any of the six populations to be significantly different in drought tolerance based on latitude, population responses were not uniform, and further study into drought tolerance is needed.

Similarly, assisted gene flow is a conservation tactic in which more resilient populations are crossbred with at-risk populations at a site (Aitken and Whitlock 2013). At its best, this process yields genetic resilience to environmental factors. However, there is also evidence for the inherent risk of outbreeding depression associated with the crossing of plants from populations insurmountable adaptive differences such as edaphic preferences (Rajakaruna 2003; Montalvo et al. 2017). Knowledge of populations' adaptations to environmental factors such as water availability can help inform decisions to effectively implement conservation management tactics such as assisted migration and assisted gene flow in a vulnerable location. Since water availability significantly impacted growth and reproductive success, site-specific details of water availability should be considered when sowing *Lasthenia* seeds for restoration to achieve maximum success.

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APPENDIX 1 SUMMARY OF LOCATION AND ENVIRONMENTAL CONDITIONS FOR CHOSEN STUDY POPULATIONS OF *L. GRACILIS*. Temperature and precipitation values are averaged over the typical *L. gracilis* growing season (February–June).
¹ Climate moisture deficit (CMD) incorporates precipitation and evaporation metrics. A higher CMD indicates an area with lower levels of precipitation and higher rates of evaporation.

Population	Latitude	Longitude	Average temp. (°C)	Average precip. (cm)	CMD ¹ (ave.)
TM	39.59691	–121.54249	15.02	105.8	63.5
HC	37.17447	–121.51714	13.44	60.2	66.3
PN	36.48411	–121.1664	13.92	36.8	97.0
CB	35.07412	–119.66115	13.88	21.0	99.6
TC	34.85118	–118.69142	14.72	31.2	91.0
AB	33.22204	–116.45735	15.06	24.4	95.1

APPENDIX 2 PERCENT GERMINATION FOR 50 SEEDS PER POPULATION PLANTED ON OCTOBER 8, 2018. The germination study was run for 18 d. Seeds from populations with sufficient germination (listed below) were immediately used for our greenhouse experiment.

Population	AB	PN	HC	TC	TM	CB
Germination rate	28	72	60	84	46	32

LATE PLANTING SHORTENS THE FLOWERING PERIOD AND REDUCES FECUNDITY IN *LASTHENIA CALIFORNICA*

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ABSTRACT

Modifications in the timing of life-history events can alter the biotic and abiotic environments experienced during an organism's lifetime. In plants, germination timing plays a critical role in relation to seasonal environmental conditions, pollinator availability, competitive dynamics, etc. Individuals can compensate for a change in timing of germination by modifying their growth and flowering time. However, biotic and abiotic factors may affect these compensatory responses. In this study we assess how biotic and abiotic differences due to planting date influence the timing of flowering, survival, and reproduction. To do this we manipulated germination timing of the annual California Goldfields (*Lasthenia californica* DC. ex Lindl., Asteraceae) in a serpentine grassland in northern California, by seeding three times during the growing season (November, January, and March). Neighbor removal plots were compared with control plots to examine influences of close neighbors and seasonal priority effects (due to early individuals pre-empting resources) on flowering time and duration, growth, and reproductive success (survival to reproduction and inflorescence production). Both planting date and neighbor removal treatments significantly impacted flowering time and duration, growth, and reproduction in this species. Later planting dates did delay flowering time, but this delay was minimal as flowering time was constrained within set biotic and abiotic boundaries. In addition, we find that a mixture of planting times and levels of neighbor removal can extend the duration of flowering on the landscape. Inflorescence production and survival declined with later planting dates, but neighbor removal counteracted this decline. We find that *L. californica* exhibits graded growth allocation, as well as plasticity in flowering time in response to planting date. This study has implications for the timing of restoration projects, as planting time influences both the timing of flowering as well as the overall reproductive success of planted individuals. Our results suggest that practitioners should aim to plant earlier in the season, but that neighbor removal may counteract some of the costs of late planting.

Key Words: competition, flowering duration, flowering time, germination time, *Lasthenia*, plant phenology, planting time, restoration.

Growth and reproductive timing are critical for individual success. This is especially true for annual plants, as an individual's lifetime reproductive output is dependent on the conditions experienced over a single growing season (Cohen 1976; Schmitt 1983). In annual species, germination timing dictates the biotic and abiotic conditions experienced throughout the lifetime of an individual, and therefore shapes an individual's fitness. Plants go through an initial juvenile phase of vegetative growth to acquire sufficient carbon reserves before flowering (Simpson and Dean 2002). Therefore, germination timing also influences reproductive timing, with implications for population dynamics and community interactions.

Germination timing may be optimized in different ways during the growing season. Earlier germinating individuals benefit from a longer growing season and early access to resources (Lortie and Turkington 2002; Wainwright et al. 2012). Later germinating

individuals may benefit from avoiding unfavorable conditions early in the season (e.g., frost) (Petrů et al. 2006; Donohue et al. 2010; Mercer et al. 2011), but may experience harsh end of season conditions, such as early frosts or, in Mediterranean-type climates, drought and heat extremes. Therefore, a population may benefit from staggered timing of germination across the season, which may also result in both inter- and intraspecific temporal resource partitioning (Dyer et al. 2008; Orrock and Christopher 2010; Leverett et al. 2018).

Plant reproductive timing is initiated as a response to abiotic factors in the environment, and flowering can be triggered by temperature, moisture and/or photoperiod cues, interacting with plant size and internal resource states (Rathcke and Lacey 1985). With sufficient resource availability, an annual plant will initiate reproductive growth after the abiotic cue(s) triggering flowering are experienced. Because

nearby plants modify the temperature, moisture, and light in the surrounding environment, as well as available resources (Rathcke and Lacey 1985; Simpson and Dean 2002), inter- and intraspecific plant-plant interactions (e.g., competition and facilitation) also play an important role in the timing of reproduction and can have strong effects on the evolution of life history timing (Ellner 1987; Metcalf et al. 2015; Leverett 2017).

In grasslands of Mediterranean-type climates, season duration is relatively unpredictable. In northern California, precipitation is highly variable, and expected to become more so as the climate changes (Swain et al. 2018). Most annual plant germination in California grasslands occurs after rainfall events of at least 15 mm (Heady 1977). Rainfall in these systems may come as early as October or as late as March, and the onset of the summer dry season is variable. For this reason, species growth and reproductive timing are often dependent on precipitation timing, sometimes in complex and dynamic ways (e.g., Pearson et al. 2021). In locations like this, with unpredictable season duration, annual plants are expected to exhibit a graded allocation strategy, in which both vegetative and reproductive growth occur simultaneously during an intermediate period between purely vegetative and purely reproductive growth (King and Roughgarden 1982). This graded allocation strategy allows for bet-hedging and is predicted to yield optimal reproductive allocation when season duration is unpredictable (Wong and Ackerly 2005). Based on this theory, we expect that plants in Mediterranean-type grasslands will exhibit a graded allocation strategy, and a capacity to respond adaptively to variation in germination or planting dates.

In environments with physical stresses that limit plant growth, such as systems with ultra-mafic soils (e.g., serpentine), the importance of competition vs. facilitation in neighboring plants can vary. Competition may be strong between species because resources are scarce (Tilman 1988), but facilitation may also play a role if neighboring vegetation can improve survival (Bertness and Callaway 1994). Because endemics to low-fertility soils are often poor competitors, these systems are generally thought to be refuges from competition (Tansley 1917; Sharitz and McCormick 1973; Kruckeberg 1984). Facilitation is predicted to be stronger in harsh environments, but the balance of competition and facilitation may vary over time, as seen in more productive systems (Leverett 2017). Serpentine soil systems are characterized by high variation in their soils, plant density and productivity, and therefore the influence of species interactions may also vary across microsites (Moore and Elmendorf 2011).

The timing and duration of flowering is also important to pollinators. Insects that rely on nectar and pollen may be especially dependent on the timing of early season flowering species as these are the first floral resources available. Bees and other pollinators

require pollen and nectar resources throughout their flight and nesting seasons, and longer flowering seasons support robust bee populations (Russo et al. 2013). The timing of plant and insect life history events have both been shifting in response to climate change (Elzinga et al. 2007), and a longer duration of flowering may support both plant and insect populations as timing shifts occur, by buffering asynchrony in the timing of interacting species (Olliff-Yang et al. 2020). Because of this, we are interested in factors that influence flowering duration.

In this study we examine the effects of differential seeding time on plant growth and flowering time, with the goal of informing restoration management practices. We explore how differences in biotic environments and abiotic conditions after germination due to different planting dates and presence vs. absence of neighbors influence the timing and duration of flowering and reproductive success in a widespread annual plant used commonly in restoration practices. Specifically, we address: 1) Does delayed planting time delay individual plant flowering time? 2) How does planting time influence population flowering duration? and 3) How does germination timing influence survival and reproduction with and without neighboring plant removal?

METHODS

Study Site and Species

The study species, California Goldfields (*Lasthenia californica* DC. ex Lindl. [Asteraceae] sensu Chan et al. 2002), is an annual forb that is widespread in northern California. This species is commonly used in restoration projects, which occur throughout the winter and early spring. As an obligate outcrossing species (Ornduff 1966), *L. californica* is dependent on pollinators for successful fruit set. This species also germinates quickly after exposure to cool wet conditions, which is ideal for establishing different germination cohorts. Populations of *L. californica* are found in a variety of different environments and habitats, including serpentine and non-serpentine grasslands.

This study occurred in a serpentine grassland at the UC Davis McLaughlin Natural Reserve in California (Lake Co., CA; 38.86007°, -122.40806°, 632 m elevation; Fig. 1A). Serpentine soils are formed from the metamorphosis of ultra-mafic mantle crust, yielding substrate that is high in heavy metals, low in essential nutrients, and low in calcium-to-magnesium ($\text{Ca}^{2+}/\text{Mg}^{2+}$) ratios (Safford et al. 2005). Site location was chosen based on the proximity to the source seed collection, and where the focal species, *L. californica*, is known to do well (C. Koheler, UC Davis Donald and Sylvia McLaughlin Natural Reserve, personal communication). This experiment was conducted during the growth season of *L. californica* during the 2016 water

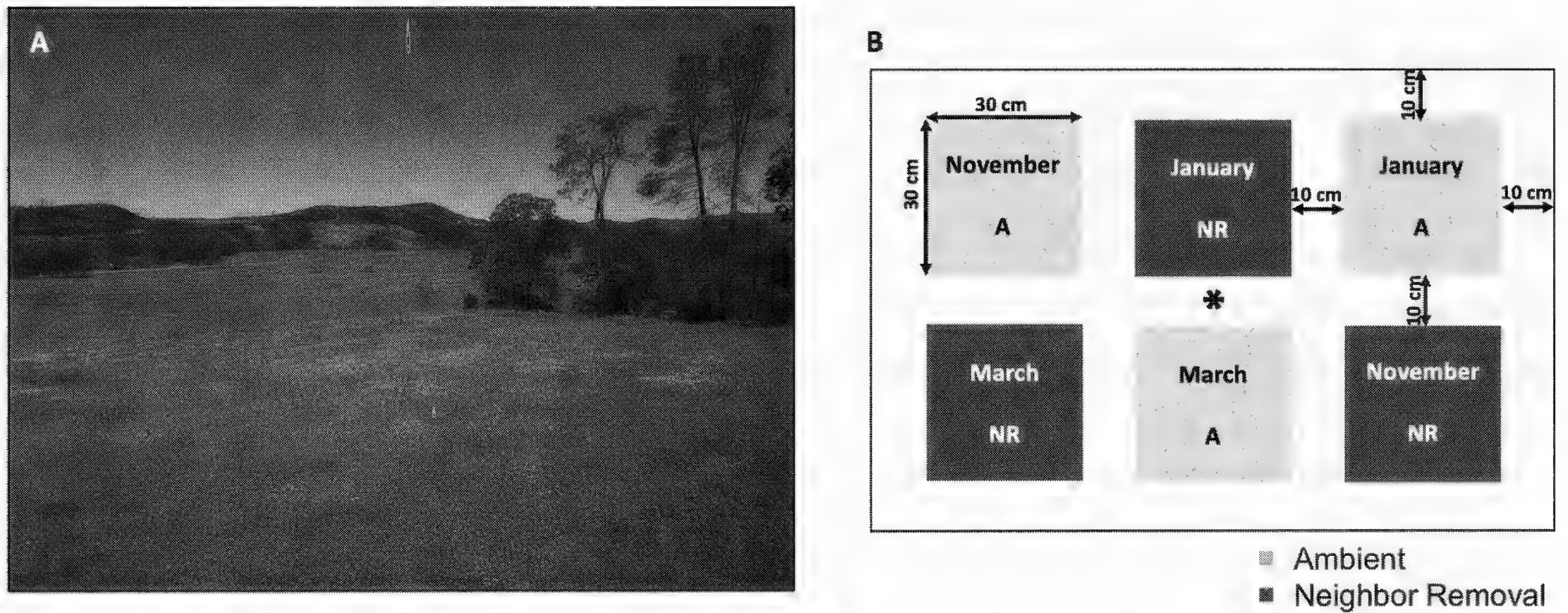


FIG. 1. Study site and experimental set up. A) Photo of study site on 15 April 2016. The south aspect of the serpentine meadow is in the foreground of the photo, and the picture was taken facing to the west. The seed collection site (“Goatgrass meadow”) can be seen in the center of the photo, and the UC NRS field station is located in the hills in the background. Photo by R. L. Olliff-Yang. B) Diagram of a single experimental block, with an example plot layout. Treatments consisted of two competition levels across three planting dates. The ibutton measuring soil temperature for each block was located in the center (asterisk). This experiment consisted of 18 of these blocks in a randomized design.

year, from November 2015 through June 2016. This species typically germinates with the first rains, grows vegetatively during the late fall and winter, and reproduces in the early spring. During this study the average daily air temperature was 12°C (minimum temperature -3.9°C , maximum temperature 32.8°C) and cumulative precipitation received was 268 mm (Western Regional Climate Center, Knoxville Creek, 38.861944° , -122.417222° , 670 m elevation [<1 km from the study site]), with a mean annual temperature of 15.9°C in 2016 (Wang et al. 2016). At this site, the average mean annual temperature was 15.2°C and mean annual cumulative precipitation was 765 mm for the 1981–2010 time period (generated with ClimateWNA v4.62, based on methodology in Wang et al. (2016)), so the conditions during the year of this study were relatively typical, but drier than the long-term mean.

Seed was collected for this study in 2011 and 2015 from many (>100) individuals in an adjacent meadow. Seeds were stored together in paper bags at room temperature in a low humidity room until they were planted. All planting dates coincided with a rainfall event (Fig. 2).

Treatments

To control for spatial variation in soil properties, treatments were set up in blocks. We set up 18 randomized blocks, each with six 30×30 cm plots arranged in a grid – one plot for each combination of planting date (3) and neighbor removal treatments (2), as described below and diagrammed in Figure 1B. Each plot had a 10 cm buffer between itself and the next plot and the edge of the block. Blocks were placed in the meadow using stratified random sampling, with nine plots placed randomly on the

north facing slope, and nine on the south facing slope of the meadow to evenly account for aspect variation. Thermochron ibuttons (iButtonLink, LLC, Whitewater, WI) were deployed in November, 5 cm below the soil surface in the center of each block, and used to calculate differences in thermal degree-days experienced by plants. These were set to record temperature every hour, and allowed us to examine relative temperature differences between blocks.

In each block two plots were seeded on each planting date: November 1, 2015, January 1, 2016, and March 1, 2016. In each plot 20 seeds were planted, with 8 and 12 seeds from the 2011 and 2015 seed collections, respectively. During planting, small holes (ca. 2 mm) were made with sewing pins and seeds were placed into the near-surface depressions, using tweezers to guide each seed in, pappus side up. Seeds were planted individually in a 4×5 grid with 5 cm distance between each other. A total of 2160 seeds were planted across all 108 plots (6 plots \times 18 blocks). The location of each planted seed was marked with a pin, with a large colorful head and pins were left in place throughout the study to identify planted seedlings. All three seeding cohorts were planted just before a rain event to stimulate germination.

In each block, one of the two plots per planting date was assigned to be an ambient plot (A), and the other a neighbor removal plot (NR) in which all other plants were clipped at the beginning of the season and every two weeks thereafter until flowering was complete (Fig. 1). Clipping at the soil surface likely did not eliminate all interactions with neighbors, but minimized these as much as possible without disturbing the soil. No plots needed to be clipped before the November planting date, as the

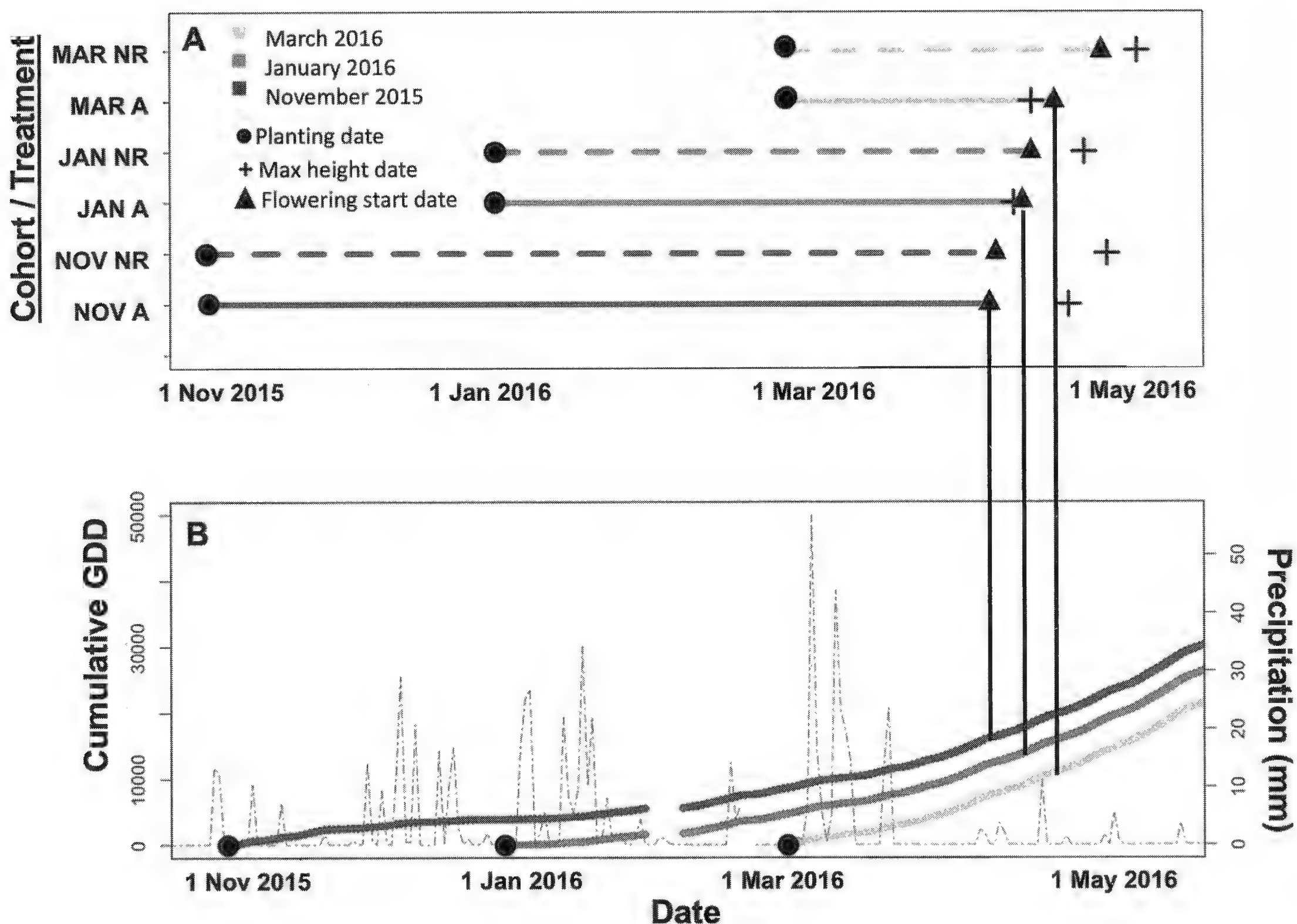


FIG. 2. Timing of biotic and abiotic events. A) Growth and flowering time of *Lasthenia californica* in experimental plots. Solid lines indicate ambient plots (A), and dashed lines indicate neighbor removal plots (NR). Circle points indicate planting dates for each cohort, plus signs note average date of maximum height reached, and triangles indicate average start of flowering. B) Growing degree day (GDD) accumulation for each cohort (solid grey lines) and precipitation events during the experiment (black dot-dash line). Large circle points at the beginning of each accumulation curve indicate planting dates in November, January, and March. In all panels colors indicate planting time (dark grey = November 1 2015, medium grey = January 1 2016, and light grey = March 1 2016). Black vertical lines connecting graphs show where flowering start corresponds with growing degree day accumulation. Note lower accumulation of degree days in both later planted cohorts.

meadow had burned in August 2015 and no germination had yet occurred.

Experimental plants were surveyed every two weeks. At each survey, vertical plant height (to tip of tallest leaf) was measured, and number of inflorescences was recorded. Survival to reproduction was calculated as the percent of germinated individuals that made it to flowering. During survey dates the inflorescence counts were categorized as “buds”, “flowering”, “recently flowering”, and “fruiting”. The “recently flowering” inflorescences were beginning to senesce (wilting and drying, with darker golden flowers and spent anthers), but could be assumed to be flowering during the week prior to the survey. Therefore, these inflorescences were given a flowering date of one week prior for the flowering time analyses. It is possible that some inflorescences were missed, going from bud to fruit in the period between surveys, but including both fresh and week-old inflorescences seemed to capture flowering in most individuals. Dates of flower counts were recorded as day of year, counting from January 1.

For each individual, start date was calculated as the date of first flower recorded, peak date as the date with most inflorescences in flower, and end date as the last date of inflorescences in flower.

Plants in the Asteraceae produce inflorescences with few to many individual flowers on a head (Keil 2021). In *L. californica*, each flower has the potential to produce one seed, although this is dependent on successful pollination. The number of seeds per inflorescence is therefore dependent on both number of flowers produced (which can range from 3 – 50+) and on successful pollination. In this study, the number of inflorescences was counted to estimate a proxy of reproduction and infer possible differences in fitness between treatments. We observed that individuals that were able to produce many inflorescences also often produced more flowers per inflorescence. Individuals with more inflorescences also had longer overall flowering duration, and therefore an increased likelihood of successful pollination events. Hence, we believe that counting inflorescences provides a conservative measure of treatment

differences in total fecundity (i.e., true differences would be even larger).

There was some difficulty with seedling identification as *L. californica* seedlings can look very similar to other species (e.g., *Plantago erecta* E.Morris before leaf hairs emerge). Although seedlings were marked by pins, if a similar species germinated very close to the pin, seedlings could have been confused. At the time of data collection, unclear species identification was noted. In addition, other *L. californica* individuals were present in ambient plots, and in some cases it was unclear which individuals were growing from the planted marked seed. All plants with uncertain identification or origin were removed from analyses.

Treatment Effects on Growth Rate

The effects of planting date, neighbor removal, and their interaction on the time from planting date to flowering were assessed using linear mixed models, using plot nested within block as a random factor ($n = 18$ blocks). Seed collection year (2011 or 2015) was also included as a fixed factor in the models to account for variation due to age of seeds. Residuals were examined to verify that model assumptions were met. Importance and significance of the fixed effects (seeding date, neighbor removal, and their interaction) in the models were determined using likelihood-ratio tests in R (version 3.31, R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Treatment Effects on Flowering Time and Duration

The effects of planting date, neighbor removal, and their interaction on flowering date (the calendar day of year of flowering in 2016), were assessed using linear mixed models, using plot nested within block as a random factor ($n = 18$ blocks), and seed collection year was included as a fixed factor to control for seed age effects. Differences in the duration of flowering in all treatment combinations (planting date \times neighbor removal) were also tested. Residuals were examined to verify that model assumptions were met. Importance and significance of the fixed effects (seeding date, neighbor removal, and their interaction) in the models were determined using likelihood-ratio tests in R.

The effect of all treatments on the overall flowering timing and duration on the landscape was also examined by looking at the differences in start and end flowering dates and assessing the flowering time overlap between treatment plots. To examine the possibilities for heterogeneous treatments on the landscape to extend overall flowering duration, we examined duration and overlap of the flowering between planting and neighbor removal treatments. Following methods in Olliff-Yang and Ackerly (2020) for examining flowering overlap, we subtracted the average end date of early treatment combina-

tions from the end dates of late treatment combinations. Similarly, early flowering start dates were subtracted from late flowering start dates. We then compared these numbers to examine season duration with and without treatment influences.

Treatment Effects on Inflorescence Number and Survival

The effects of seeding date and neighbor removal on inflorescence production were also assessed using linear mixed models, using plot nested within block as a random factor, and seed collection year was again included as a fixed factor to account for seed age. Data for inflorescence production was analyzed using a Poisson distribution generalized mixed model to fit this positive, integer-valued response variable. Due to low survival in the March cohort, reproduction differences were only tested in November and January cohorts. Fixed effect importance in the models were determined via examination of F -values from the model output. All analyses were performed in R and mixed models were fit using the *lme4* package (Bates et al. 2015).

RESULTS

Treatment Effects on Growth Rate

Planting date influenced growth rate and time to flowering. Initiation of first flower occurred faster in plots with later planting dates, as the number of days from planting to first flower was significantly reduced (Likelihood-ratio, $\chi^2_{(2,484)} = 316.2$, $P < 0.0001$, Figs. 2, 3), with approximately 52 fewer days from germination to flowering in the November vs. March planting cohorts. Neighbor removal did not influence the number of days from planting to first flower (Fig. 3A). The initiation of flowering seemed to require a minimum height threshold around 3 cm, as both January and March cohorts began to flower around this height, while November cohorts began flowering at a slightly taller height on average (Fig. 3B). Maximum height was reached at a similar time despite different planting dates (Fig. 2A).

Treatment Effects on Flowering Time and Duration

Planting date and competition removal both influenced the timing of flowering in the season. Later planting dates yielded later start, peak, and end dates of flowering in the year (Table 1), with individuals planted January 1 flowering a mean of six days later, and March 1 flowering a mean of 14 days later than individuals planted in November. While neighbor removal plots exhibited similar flowering start dates, removing neighbors shifted flowering peak and end dates later (Table 1). Both later planting time and presence of neighbors shortened flowering duration (Table 1, Fig. 3C).

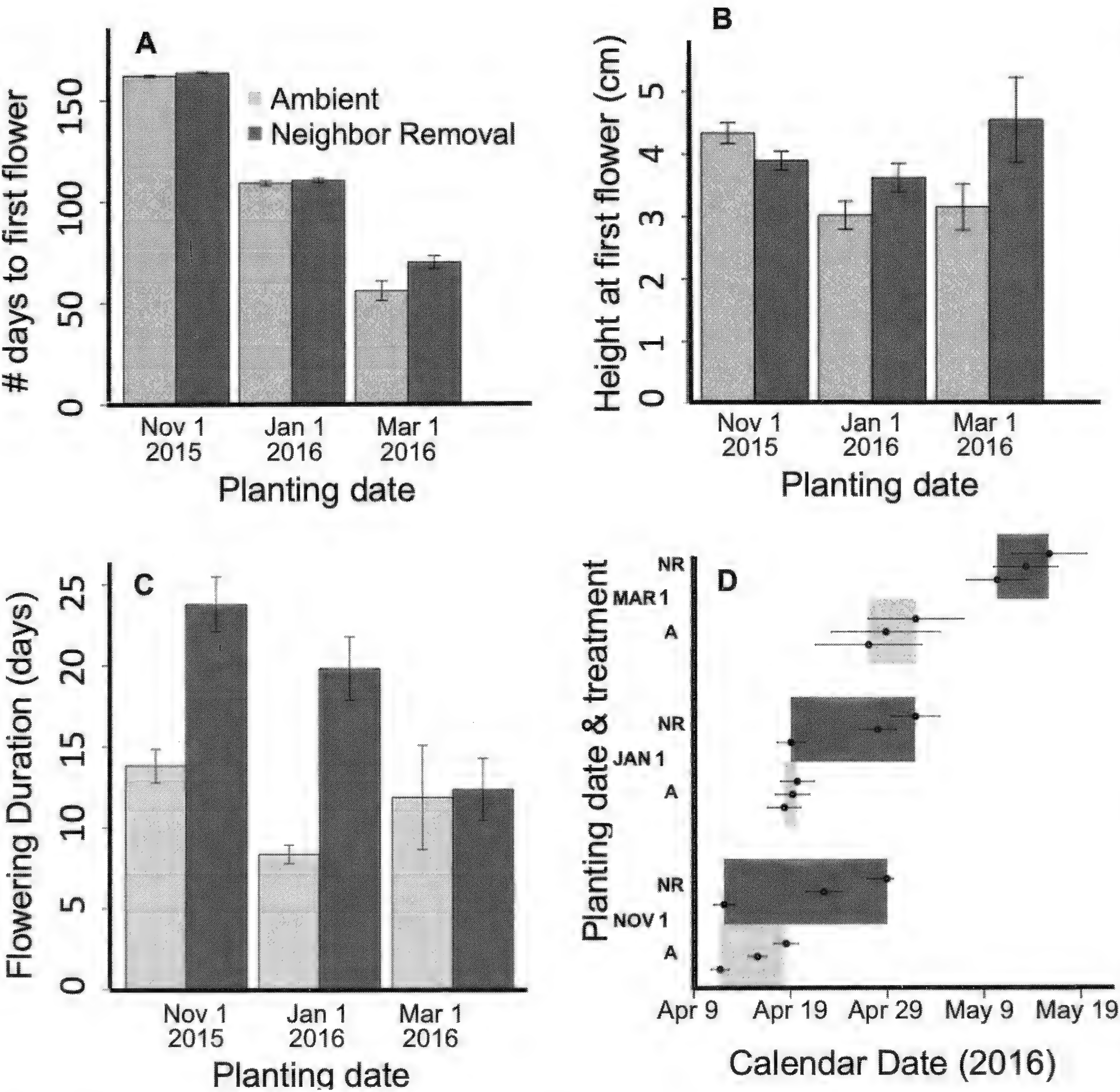


FIG. 3. Flowering phenology, duration, and season extension. Planting cohorts are indicated by month abbreviations (Nov 1 = November 1 2015, Jan 1 = January 1 2016, and Mar 1 = March 1 2016), and treatment is shown with color (neighbor removal [NR] = dark grey, and ambient [A] = light grey). In all panels whiskers indicate standard error. A) Mean number of days to first flower compared across planting cohorts and neighbor removal treatments, (Planting date significant [$F_{2, 62} = 2032$, $P < 0.0001$]) B) Mean height at first flower compared across planting cohorts and neighbor removal treatments, C) Mean flowering duration (in # of days) of individuals in each treatment and planting cohort. D) Mean start, peak, and end dates of flowering in each planting cohort and treatment. Bar ends indicate start (left end) and end (right end) dates. Middle points are peak dates. Points indicate means and are staggered vertically for visibility, with start point the lowest, and end point the highest. Whiskers show standard error.

Flowering time of plots with different treatments was complementary and planting both early and late cohorts led to longer flowering on the landscape overall, due to asynchronous flowering time between plots. Having staggered germination timing increased the duration of flowering on the landscape by approximately 11 days in ambient plots, due to different flowering time between cohorts (Fig. 3D). Flowering duration was further increased when competition removal plots were also considered, with

an increase of up to 26 days of flowering on the landscape with the staggered timing between both later planting and competition removal plots (Fig. 3D).

Treatment Effects on Survival and Inflorescence Number

The percentage survival of germinated individuals to flowering was different between planting cohorts,

TABLE 1. PLANTING TIME AND NEIGHBOR REMOVAL INFLUENCES ON FLOWERING DATE. Models of Start, Peak and End dates of flowering in 2016, as well as the Duration of flowering (calculated as the number of days flowering). Plot nested within block is included as a random effect in all models. The P-values indicate significance of including each fixed effect in the model ($\alpha < 0.05$), as determined by Likelihood-ratio Tests comparing the full model against a model with the fixed effect removed. Fixed factors include: PD = planting date (levels: November 2015, January 2016, March 2016), NR = neighbor removal (levels: ambient, neighbor removal), and CY = seed collection year (levels: 2011, 2015).

Measure	Factor	Parameters	SumSq	Mean SQ	<i>F</i>	P-value
Start	PD	2	5101.3	2550.65	50.71	< 0.0001
	NR	1	107.2	107.16	2.13	NS
	CY	1	482.9	482.93	9.60	0.002
	PD × NR	2	256.2	128.1	2.55	NS
Peak	PD	2	4083.8	2041.9	27.22	< 0.0001
	NR	1	3292	3292	43.89	< 0.0001
	CY	1	130.6	130.6	1.74	NS
	PD × NR	2	194.2	97.1	1.29	NS
End	PD	2	3538.9	1769.4	19.94	< 0.0001
	NR	1	8267.3	8267.3	93.17	< 0.0001
	CY	1	106.4	106.4	1.20	NS
	PD × NR	2	86.1	43.1	0.48	NS
Duration	PD	2	1087.3	543.7	6.00	0.0005
	NR	1	10629.1	10629.1	117.25	< 0.0001
	CY	1	128.7	128.7	1.42	NS
	PD × NR	2	581.5	290.7	3.21	0.039

with higher survival overall in earlier cohorts (Fig. 4A). Reproductive output (in November and January) was affected by both planting date and neighbor removal treatments. Later planting reduced the number of inflorescences produced by an average of 0.5 inflorescences per plant in ambient plots (Poisson, $F_{(1,9798)} = 46.56$, $P < 0.001$); although low survival in March cohort meant we were unable to test, there does not appear to be a difference between reproductive output in January vs. March cohorts in ambient plots for plants that survived to flower (Fig. 4B, light grey [ambient] bars). In neighbor removal plots, planting date appears to have only reduced inflorescence number in the March cohort, by an average of one inflorescence per plant (Fig. 4B).

Neighbor removal increased reproduction (Poisson, $F_{(1,9888)} = 69.4$, $P < 0.001$, Fig. 4B). When combined into overall inflorescences per seed planted (which incorporates germination percentage, survival to flowering and inflorescence production) the results reveal a decrease in overall inflorescence production with each planting date, and an increase with neighbor removal (Fig. 4C). Assuming that each inflorescence has at least one flower and one successful pollination event leading to a mature seed, the mean of ~1 inflorescence per planted seed in the ‘control treatment’ (early cohort with neighbors present) suggests a stable or growing population at this site, based on the demography in this study year.

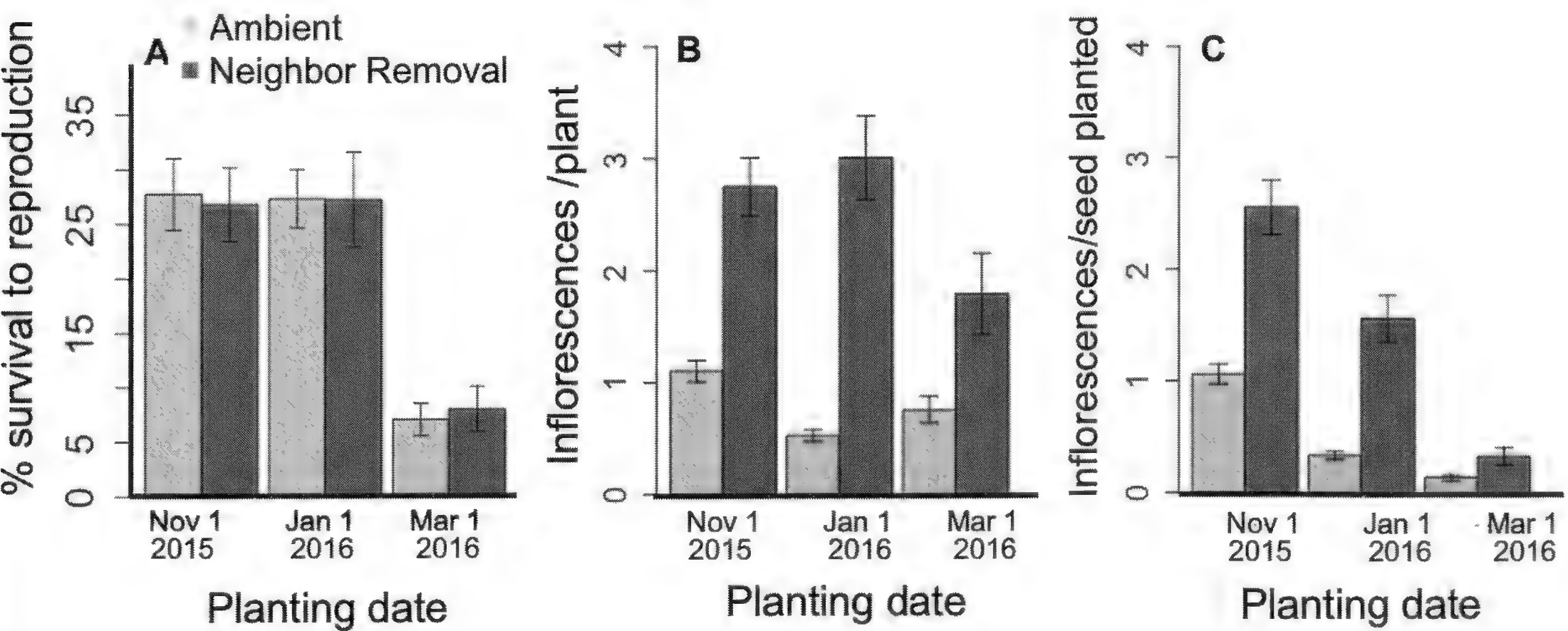


FIG. 4. Planting date and neighbor removal effect on survival and reproduction. A) percent of germinated individuals that survived to flowering, B) total inflorescence numbers per plant, and C) mean inflorescences per seed planted. In all panels light grey bars show ambient plots, and dark grey bars are neighbor removal plots. Whiskers show standard error.

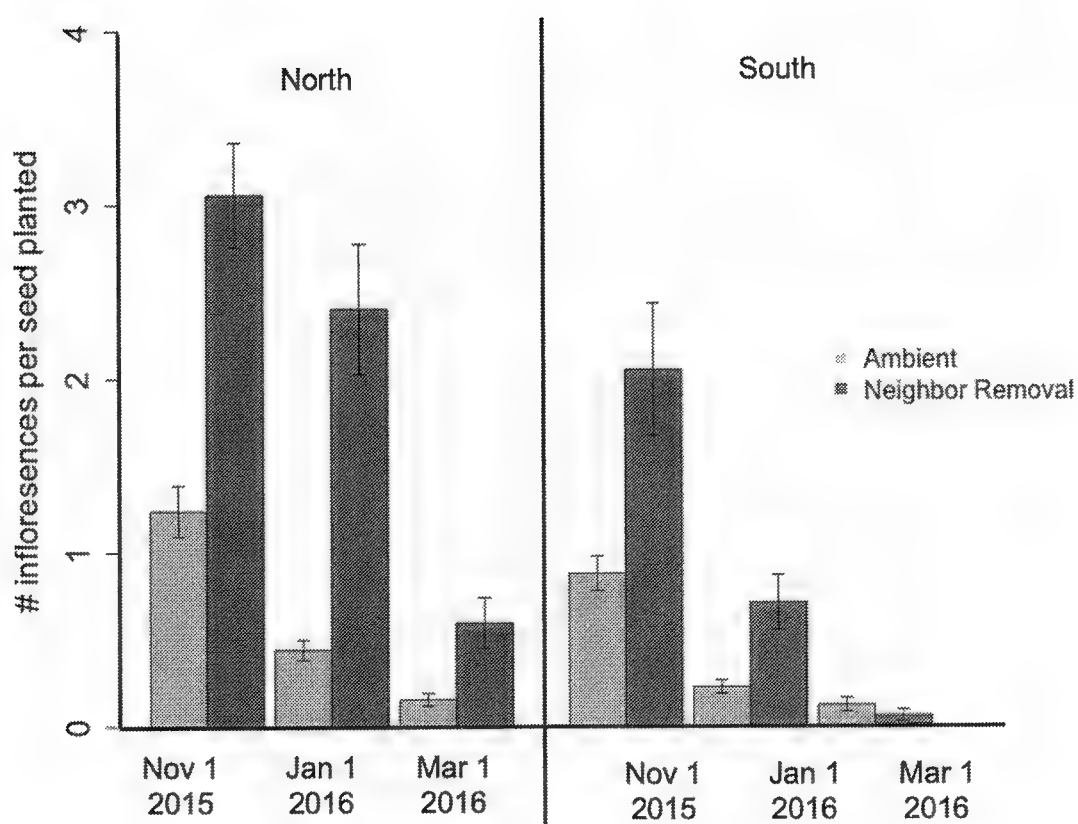


FIG. 5. Effect of neighbor removal and planting time on overall survival and inflorescence production depends on aspect. Overall inflorescence production per seed on north-facing (left panel) and south-facing (right panel) slopes. Light grey bars show ambient plots, and dark grey bars are neighbor removal plots. Whiskers show standard error

Although the topographic treatment was not replicated, aspect did influence survival and reproduction in the treatments. Plots on the north-facing slope exhibited higher overall reproduction (Fig. 5), as survival and inflorescence production were lower in general on the south aspect. Neighbor removal seemed to ameliorate some of the effects of late planting on fecundity, except for the latest planting date (March 1) on the south-facing slope.

DISCUSSION

Planting time and neighbor removal influence flowering time, flowering duration, and inflorescence number in *L. californica*. This study gives evidence that flowering in this species responds to planting date, with delayed peak and end flowering dates and a shorter duration of flowering in later germinating individuals. Flowering time was strongly tied to a window in spring, and months of difference in planting time only led to slight differences in the calendar date of flowering time. Additionally, later planting dates decreased the survival and reproduction of individuals, resulting in lower overall planting success of later cohorts. The longer duration of flowering observed in early germinating and neighbor removal plots was due to an increase in the number of inflorescences produced by individual plants. We recommend earlier plantings with density reduction treatments for the most successful individuals in restoration contexts and suggest that extremely late planting should be avoided unless there is a goal to extend flowering at the end of the season.

Patterns observed in days to flower and date of maximum height reached revealed nuances of growth allocation timing. Despite the extreme differences in germination time, flowering start time was not shifted as dramatically as might be expected, indicating that

that abiotic constraints at the end of the season may limit the capacity for flowering time shifts. Additionally, plants compensated for the germination delay by speeding up development, and maximum height was reached on similar dates in all planting cohorts (Fig. 2). Later planting dates also shortened the number of days between germination and first flower (Fig. 3A). A similar phenomenon has been noted in frog development with later egg hatch cohorts exhibiting faster development in the absence of priority effects (Murillo-Rincón et al. 2017). The shortened time to reproduction reveals the tradeoff between vegetative growth and reproduction at the end of the season, likely due to increasing water limitation as summer approaches. Neighbor removal seemed to ameliorate this tradeoff slightly, as plants were able to begin flowering slightly later and produce more inflorescences in these plots (Figs. 3A and 4B), indicating that competitive effects are stronger than any facilitative interactions among neighboring plants. However, despite faster growth rates, there seemed to be a height threshold at the time of first flower, and flowering in the March cohort was delayed until individuals reached a height of approximately 3 cm (Fig. 3B). This suggests that the switch to flowering is not entirely dependent on environmental cues, and this height may reflect a size threshold where sufficient carbon accumulation for reproduction has been reached.

These data show that *L. californica* exhibits a graded allocation growth strategy, and a capacity to respond to variation in germination or planting dates. In fact, over 75% of flowering individuals grew vegetatively after initiation of reproductive growth (464 of 588 [78.9%] individuals grew in height after bud initiation). Additionally, as flowering start occurred at a similar time in all cohorts despite drastically different germination dates, this species exhibits plasticity in flowering time, switching from vegetative growth to flowering when the right conditions occur, as long as sufficient plant size has been achieved.

The season length in California can vary dramatically each year due to rainfall patterns and climate oscillation patterns (e.g., El Niño Southern Oscillation), and differences in year-to-year rainfall are becoming more extreme and variable (Swain et al. 2018). The shallow and infertile soils in serpentine grasslands typically hold little water, and seasonal flowering time in these systems is often earlier due to faster soil dry-down rates (Schmitt 1983; Rajakaruna and Bohm 1999; Rossington et al. 2018). These factors make the season length unpredictable, with plant senescence at the end of the season tied to the soil dry-down. Therefore, a graded-allocation strategy is a logical response to natural selection, as it allows for bet-hedging when season lengths are unpredictable (Wong and Ackerly 2005).

Delayed planting greatly reduced the number of inflorescences produced, but neighbor removal ameliorated this effect. The decrease in reproduction

occurred between November and later cohorts in ambient plots, but did not decline until the March cohort in neighbor removal plots. This pattern indicates that competition removal may ameliorate some loss of fitness due to late planting. Early growth was best for reproductive output, and therefore early planting time should be prioritized in restoration projects. This difference in total inflorescence production may indicate fitness differences, but full reproductive fitness will depend on total seed set. *Lasthenia californica* is obligately outcrossing (Ornduff 1966), and fluctuations in the presence of pollinators throughout the season might further influence reproductive success. It should also be noted that there was less germination overall in the March planting cohort, but seeds planted late may survive in the soil and yield successful individuals in the next growing season, and populations may vary in seed dormancy (e.g., congener *L. fremontii* (Torr. ex A.Gray) Greene [Torres-Martínez et al. 2017]). Tracking cohorts over multiple years is needed to determine any long-term impacts of planting time.

Flowering duration was shortened by later planting time, but neighbor removal influenced this pattern (significant PD \times NR interaction, $F_{(2,106)} = 3.7$, $P = 0.03$). Neighbor removal lengthened individual plant flowering duration in the November and January, but not the March, cohorts (Fig. 3C). This interaction between planting time and neighbor removal treatments suggests that flowering at this location may be more constrained by abiotic factors at the end of the season (high temperature and low moisture) than at the beginning of the season. Flowering duration was cut short due to constraints at the end of the season regardless of neighbor environment. This likely reflects the stress of elevated temperatures and drought as summer approaches in Mediterranean-type systems, which is the main constraint for survival and reproduction of annual plants in these regions (Larcher 2000). This finding is consistent with congener *L. gracilis* (DC.) Greene, as individuals flower for longer periods of time in higher moisture conditions (Cox and Olliff-Yang 2021).

Neighbor removal delayed peak and end flowering, an effect that was most pronounced in the March cohort (Fig. 3D). Although reduced competition resulted in larger plant sizes overall, this did not result in an earlier switch to flowering, as found in other systems (Rathcke and Lacey 1985). This supports the prediction that selection should favor individuals that accelerate flowering in the face of increasingly scarce resources (Callahan and Pigliucci 2002), although only in peak and end flowering. The fact that flowering start date was not different with neighbor removal, indicates that neighbor removal indirectly contributes to the later peak and end dates, likely due to increased plant size and higher numbers of inflorescences produced per plant in plots with fewer competitors (Figs. 3B and 4B).

The study meadow burned in August 2015, only a few months before this experiment was conducted.

Light availability is unlikely to have been changed much, as this is a low-productivity system with no canopy cover, but any soil nutrient changes from the fire might have an effect. Concentrations of calcium, potassium, and phosphorus are enriched in ash due to their resistance to volatilization (Pellegrini and Jackson 2020), and availability of these nutrients often increases in the soil after a fire. The low soil $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio often limits growth in serpentine soils, and fire can increase $\text{Ca}^{2+}/\text{Mg}^{2+}$ in these systems (DeSiervo et al. 2015). These nutrient changes may affect growth and plant-plant interactions, as species take advantage of the nutrient pulse. Fire has been shown to enhance native species richness on serpentine soils in this area (Harrison et al. 2003). While fire is likely to have some influence on the growth responses in this study, fire effects are often less pronounced on serpentine than more productive soil types (Safford and Harrison 2004).

Theory predicts that longer flowering duration can potentially ameliorate impacts of phenological mismatch on pollination mutualisms with climate change (Olliff-Yang et al. 2020). Therefore, based on our results, staggered reproductive timing in one location due to seeding time and competition reduction may also be a valuable conservation technique. Variable seeding time extended the flowering time in *L. californica* by an average of 11 days in ambient plots. Competition reduction in neighbor removal treatments yielded an average extension of 13 days. The extension from competition removal occurred mainly due to a lengthened end of season flowering duration, as individuals continued to produce inflorescences. Combining the two techniques in this location resulted in an average overall flowering time extension of 26 days. Therefore, these techniques may aid in increasing the overlap between flowering and pollinator presence, extending pollen and nectar resources for mutualistic flower visitors and enhancing reproductive assurance for the plants. However, the tradeoff of reduced survival and reproductive effort of later planted individuals should be taken into consideration.

During the study we observed additional heterogeneity in flowering time and reproduction due to aspect. The blocks were evenly split between a north-facing and south-facing slope, and abiotic differences likely led to additional extension in the duration of the flowering season (e.g., Olliff-Yang and Ackerly 2020). Plots on the north-facing slope exhibited later and longer flowering on average. Aspect also influenced inflorescence production (Fig. 5), interacting with the effect of planting time. Survival and inflorescence production were lower in general on the south aspect, and competition removal did not seem to ameliorate the effects of the March planting time. Although the topographic treatment was not replicated, this observation suggests that the success of later planting will depend on the abiotic conditions of a site. There may be a longer window of time to plant in cooler and wetter habitats, such as on North-

facing slopes, and planting in hotter drier parts of the landscape should be prioritized earlier in the season.

In this study we see that both planting date and plant-plant interactions can influence the timing and success of reproduction. While there is a capacity to respond to late planting, as *L. californica* exhibited both plasticity in flowering time and a graded allocation growth strategy, later germination timing reduced fecundity and shortened flowering duration of individuals. This study has implications for the timing of grassland management and restoration practices, as planting time and density changes (e.g., due to grazing) may influence both the timing of flowering and overall fitness of individuals. Restoration practices in California annual grasslands include planting seed from October through March. These results suggest that later planting may result in an unsuccessful growth season. Reduced competition can ameliorate some of the negative impacts of a late start, but earlier planting dates should be prioritized.

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PHENOLOGICAL SENSITIVITIES TO CLIMATE ARE SIMILAR IN TWO *CLARKIA* CONGENERS: INDIRECT EVIDENCE FOR FACILITATION, CONVERGENCE, NICHE CONSERVATISM, OR GENETIC CONSTRAINTS

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ABSTRACT

To date, most herbarium-based studies of phenological sensitivity to climate and of climate-driven phenological shifts fall into two categories: detailed species-specific studies vs. multi-species investigations designed to explain inter-specific variation in sensitivity to climate and/or the magnitude and direction of their long-term phenological shifts. Few herbarium-based studies, however, have compared the phenological responses of closely related taxa to detect: (1) phenological divergence, which may result from selection for the avoidance of heterospecific pollen transfer or competition for pollinators, or (2) phenological similarity, which may result from phylogenetic niche conservatism, parallel or convergent adaptive evolution, or genetic constraints that prevent divergence. Here, we compare two widespread *Clarkia* species in California with respect to: the climates that they occupy; mean flowering date, controlling for local climate; the degree and direction of climate change to which they have been exposed over the last ~115 yr; the sensitivity of flowering date to inter-annual and to long-term mean maximum spring temperature and annual precipitation across their ranges; and their phenological change over time. Specimens of *C. cylindrica* were sampled from sites that were chronically cooler and drier than those of *C. unguiculata*, although their climate envelopes broadly overlapped. *Clarkia cylindrica* flowers ~ 3.5 d earlier than *C. unguiculata* when controlling for the effects of local climatic conditions and for quantitative variation in the phenological status of specimens. However, the congeners did not differ in their sensitivities to the climatic variables examined here; cumulative annual precipitation delayed flowering and higher spring temperatures advanced flowering. In spite of significant spring warming over the sampling period, neither species exhibited a long-term phenological shift. Precipitation and spring temperature interacted to influence flowering date: the advancing effect on flowering date of high spring temperatures was greater in dry than in mesic regions, and the delaying effect of high precipitation was greater in warm than in cool regions. The similarities between these species in their phenological sensitivity and behavior are consistent with the interpretation that facilitation by pollinators and/or shared environmental conditions generate similar patterns of selection, or that limited genetic variation in flowering time prevents evolutionary divergence between these species.

Key Words: *Clarkia cylindrica*, *Clarkia unguiculata*, climate change, herbarium, phenology, species divergence.

The relationship between phenological events — particularly the onset of flowering — and local climatic conditions has recently attracted the intense interest of ecologists and evolutionary biologists for several reasons (Wolkovich et al. 2012; Willis et al. 2017a). First, quantitative estimates of the sensitivity of flowering date to geographic variation in chronic climatic conditions or to inter-annual variation in climate over the last century can be used to forecast species-specific shifts in flowering date in response to upcoming climate warming. These predicted phenological shifts may, in turn, be used to infer changes in the exposure of flower buds or newly opened flowers to environmental stressors such as early spring frost (Park et al. 2020). Second, observed differences in sensitivity to climatic changes among sympatric or regionally co-occurring species lead to the expectation that community-level flowering patterns will change in response to a changing climate (Park and

Mazer 2019). Similarly, differences in the sensitivities of co-occurring native and invasive plants provide insights into the causes of their relative success (Willis et al. 2010). Third, phenological shifts that result in asynchronous activity between mutualistic species (e.g., plants and their pollinators) or between plants and their antagonists (e.g., herbivores, florivores, or fungal spore vectors) may affect individual fitness or population mean fitness of all interacting taxa, and such mismatches may have cascading effects throughout an ecosystem (Kudo and Ida 2013; Kudo and Cooper 2019).

Detecting species-specific or multi-species phenological responses to climatic conditions requires observations that capture variation in both phenological behavior (comprising the response variable) and climatic parameters or other predictor variables, such as elevation, latitude, and longitude, that may serve as a proxy for climate. Given this requirement,

herbarium collections are highly suited to the study of phenological variation and its environmental correlates for species that have been widely collected over space and time (Yost et al. 2019; Pearson 2019; Pearson et al. 2020, 2021). This suitability applies particularly well to annual forbs, for which herbarium specimens often contain intact individuals whose phenological status may be scored in a highly quantitative manner, thereby capturing the precise phenological condition of the entire individual plant when collected (Willis et al. 2017; Love et al. 2019; Goëau et al. 2020).

To date, most herbarium-based studies of phenological sensitivity to climate fall into one of three non-mutually exclusive categories. The first is represented by detailed studies of individual species in which the sensitivity to long-term mean climatic conditions and/or to inter-annual variation in climate is estimated, and some report interactions between predictor variables that affect phenological behavior (Robbirt et al. 2011; Gaira et al. 2011, 2014; Matthews and Mazer 2016; Ellwood et al. 2019; Love et al. 2019; Petruski et al. 2019; Banaszak et al. 2020; Pearson et al. 2021). The second category comprises synthetic studies of multiple species and higher taxa, aiming to detect general similarities and differences among taxa or communities with respect to their phenological responses to climatic factors that vary over time or space (Primack et al. 2004; Miller-Rushing et al. 2006; Houle 2007; Gallagher et al. 2009; Diez et al. 2012; Panchen et al. 2012, 2017; Diskin et al. 2012; Calinger et al. 2013; Li et al. 2013; Mazer et al. 2013; Hart et al. 2014; Park 2014; Davis et al. 2015; Kharouba and Vellend 2015; Rawal et al. 2015; Munson and Long 2017; Park and Schwartz 2018; Jones and Daehler 2018; Park et al. 2018; Park and Mazer 2018, 2019; Berg et al. 2019; Pearson 2019; Kopp et al. 2020; Park, Ramirez Parada, and Mazer 2020; Reeb et al. 2020). A few studies in both categories have begun to investigate sources of intra-specific variation in phenological sensitivity (Matthews and Mazer 2016; Park et al. 2018; Song et al. 2020). The third category is represented by studies that compare the sensitivities derived from herbarium specimen data to those derived from high-resolution field observations (Robbirt et al. 2011; Davis et al. 2015; Zohner and Renner 2014) or from remotely sensed data (Park 2012) to determine the reliability of the former, which are known to include sampling biases of various kinds (taxonomic, geographic, seasonal, and temporal; Daru et al. 2017).

Few herbarium-based studies, however, have compared phenological sensitivities to climate of closely related species that are broadly sympatric and that share pollinators. Similarities between such species in phenological sensitivity to local climatic conditions might be expected due to several non-mutually exclusive processes or factors, including: (a) phylogenetic niche conservatism, whereby closely related species “inherit” their exposure to similar environmental conditions, resulting in parallel evo-

lution of mean flowering date and/or sensitivity to the same environmental cues used to induce flowering; (b) the evolution of synchronous flowering because of its advantage in attracting a shared set of pollinators or in satiating predators; or (c) a lack of genetic variation in phenological sensitivity, precluding its evolutionary divergence between taxa. The use of herbarium specimens to compare congeners with respect to flowering time and climate-driven phenological sensitivity therefore provides an opportunity to address several fundamental ecological and evolutionary questions, including the following:

- (1) Do congeners differ in the timing of flowering, controlling for spatiotemporal variation in local environmental variables that might influence flowering time, such as elevation, temperature, and precipitation? Closely related species that share pollinators and that exhibit overlapping geographic distributions may be expected to evolve to differ in mean flowering time when exposed to the same climatic conditions as a result of divergent natural selection favoring genotypes that avoid direct competition for pollinators or that minimize heterospecific pollen transfer (Rathcke and Lacey 1985; Fenner 1998). In such cases, divergent flowering times would contribute to reproductive isolation, reducing the potential for hybridization and/or heterospecific pollination, both of which may reduce seed production or quality (Morales and Traveset 2008; Moreira-Hernández and Muchhala 2019; Ashman and Arceo-Gómez 2013; Arceo-Gómez et al. 2019), particularly when heterospecific pollen transfer occurs between close relatives (Streher et al. 2020).
- (2) Independent of mean flowering times, do closely related taxa exhibit distinct *sensitivities* of flowering time to local temperature and precipitation? Close relatives that share habitats and pollinators might be expected to evolve contrasting or opposing phenological responsiveness to a given set of environmental cues because, just as is the case for mean flowering date, divergence in sensitivity may promote reproductive isolation and reduce heterospecific pollen transfer. Alternatively, species that share pollinators might be expected to evolve to use the same environmental cues to induce flowering where synchronous flowering attracts more pollinators per capita or per flower due to potential synergistic effects of producing a large floral display at the community level (McGuire 1993; Kudo 2006; Sargent and Ackerly 2008).
- (3) Does the flowering phenology of closely related species respond similarly to geographic variation in long-term mean climatic conditions and to inter-annual variation in local climate? The process that generates associations between flowering date and long-term mean climatic conditions may differ from the process (or processes) that causes flowering date to respond to inter-annual variation in climate. Strong associations between long-term, chronic, local climatic conditions and flowering date likely represent the outcome of natural selection, while phenological shifts in response to comparatively unpredictable inter-annual variation may represent adaptive or non-adaptive plastic responses to short-term environmental variation or, potentially, the effects of rapid adaptive evolution. Given that different processes (adaptation vs. plasticity) may contribute to phenological sensitivity to long-term vs. inter-annual

climatic conditions, both the magnitude and the direction of these sensitivities may differ within or between species (Mazer et al. 2020).

In the current study, we address these questions using physical herbarium specimens of *Clarkia cylindrica* and *C. unguiculata* (Onagraceae), a pair of ecologically similar annual herbs native to California. To determine whether these *Clarkia* congeners respond similarly to climate or to climate change, we tested for differences between them with respect to the climatic conditions they occupied; estimated the degree of climate change they experienced in their sampled ranges during the past century; used phenoclimatic models to estimate the phenological responsiveness (i.e., sensitivity) of each species to both long-term mean climate and inter-annual variation in climate; and tested for long-term shifts in the estimated flowering date over the past century in both species.

METHODS

Overview

This study comprised five steps. First, we sampled reproductive specimens of each species from throughout its range to record each specimen's date and site of collection, as well as a quantitative estimate of its phenological status, which ranged from bearing only flower buds to bearing only ripe fruits. Second, we compared the climatic conditions occupied by each species to assess their habitat preferences and tested for species differences in mean flowering date independent of local, long-term climatic conditions. Third, we used linear models to detect the degree of climate change that each species experienced over the past 112–119 yr across its sampled range (1900–2012 for *C. cylindrica* and 1892–2011 for *C. unguiculata*). Fourth, we constructed and tested phenoclimatic models to detect the effects of local mean maximum spring temperature (Spring Tmax) and annual precipitation (AP) on each species' flowering date, which was estimated as the day of the year (DOY) on which a reproductive specimen was collected; these linear models also controlled statistically for each specimen's phenological status (estimated using a quantitative index of its reproductive progression; Love et al. 2019).

The phenoclimatic models tested here provide measures of the sensitivity of flowering phenology to climatic conditions estimated at two temporal scales: decadal and year-of-collection. Specifically, each site of specimen collection was characterized by its mean climatic conditions from 1921–2010 (i.e., long-term climate, or climate “normals”), and by the *deviation* between climatic conditions at the site in the year of collection and the site's mean long-term climate conditions (i.e., climate *anomalies* due to inter-annual variation in temperature and annual precipitation [AP]). When both kinds of parameters are included as explanatory variables in linear models designed to

predict the DOY of flowering, the sensitivity of DOY to climate normals reflects a combination of local adaptation and plastic responses to spatial variation in chronic climatic conditions, while phenological responsiveness to interannual variation is due primarily or wholly to plastic responses to short-term local conditions. Other investigators have used this approach, including Mazer et al.'s (2020) study of seed size variation in *Clarkia*; Pearson et al.'s (2021) investigation of *Eschscholzia californica* (California Poppy; Papaveraceae), and Parker's (2021) study of five species of *Arctostaphylos* (Ericaceae) and *Ceanothus* (Rhamnaceae).

In all models, we controlled for variation in the phenological status of specimens, which can confound the relationship between a specimen's collection date and the actual date of flowering onset. These models were then used to compare the direction and magnitude of the two species' phenological sensitivities to local temperature and precipitation normals and anomalies, and to test for interactions between climate variables that may have influenced flowering time. Finally, we tested for phenological shifts in estimated flowering date within each species during the ~115-yr sampling period to determine whether long-term temporal trends in flowering phenology were consistent with each species' sensitivity to inter-annual variation in temperature and precipitation, and with the degree of climate change that each species experienced.

Study Species

Clarkia is a well-studied genus of ~40 species of self-compatible, annual, herbaceous wildflowers native to the western U.S. (Lewis and Lewis 1955). Wherever they occur, populations of *Clarkia* are among the last spring wildflowers to bloom (typically flowering in May or June), and they produce dense and showy floral displays. The two taxa selected for the current study — *Clarkia cylindrica* ssp. *clavicaarpa* W. Davis (section Peripetasma) and *C. unguiculata* Lindley (section Phaeostoma) — inhabit open or disturbed habitats and roadsides in the foothills, grasslands, and oak/pine woodlands of the Coastal Ranges, Transverse Ranges, and Sierra Nevada in California. These taxa are adapted to a Mediterranean climate, although the sites sampled for the current study vary widely with respect to long-term conditions. Among sites, long-term MAP estimated from 1921–2010 ranges from 141–1377 mm, and mean spring Tmax for the same period ranges from 9.9–24.9°C. *Clarkia cylindrica* has been described as “normally outcrossing” (Davis 1970), and *C. unguiculata* is predominantly outcrossing, although populations of the latter differ in their outcrossing rates (Vasek 1958; Hove et al. 2016; Ivey et al. 2016). Both species are restricted to California, are typically found at elevations below 1500 m, and are diploid ($n = 9$).

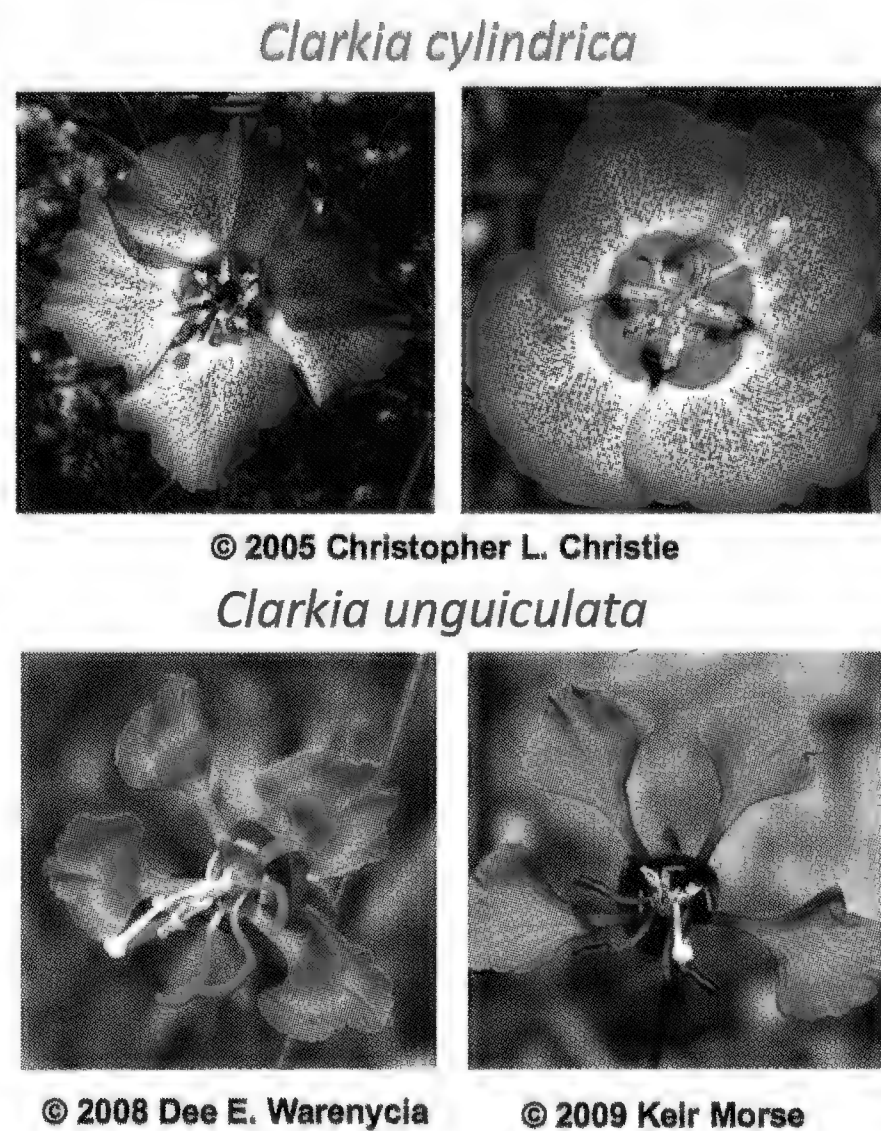
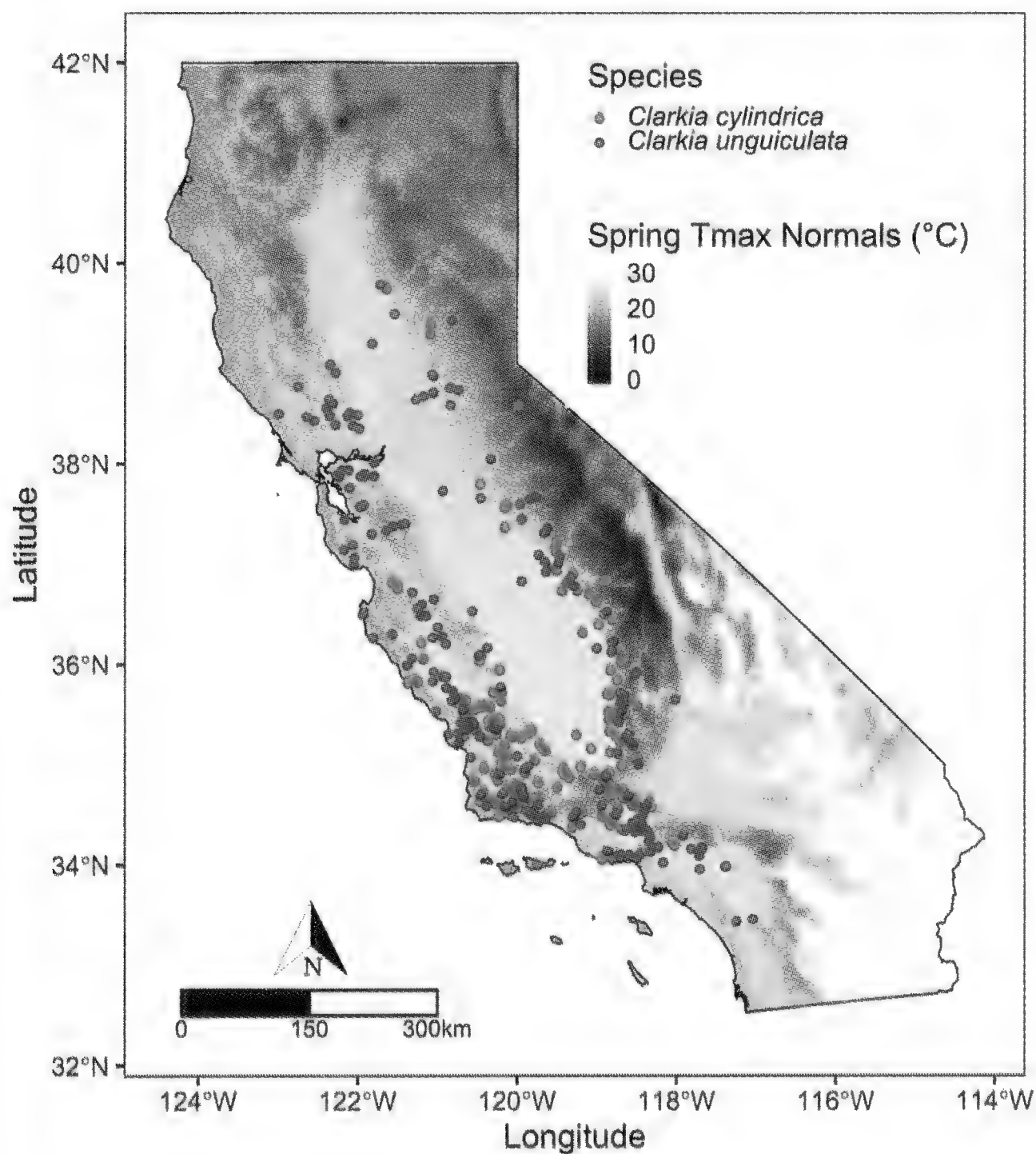


FIG. 1. The distributions of the collection sites of the specimens sampled for the current study, and photos of the two focal species examined here: *Clarkia cylindrica* and *C. unguiculata*.

The two species have been found to co-occur regularly in the southern Sierra Nevada (Moeller 2004), but they are also found with other congeners. Lewis and Lewis (1955) reported that, at the time of the publication of their 1955 monograph on *Clarkia*, *C. unguiculata* and *C. cylindrica* could be found in mixed colonies with, respectively, any of 17 or 10 other *Clarkia* species (including each other). So, the potential for these species to compete for pollinators or to facilitate the pollination of congeners is not restricted to their interactions with one another. In addition, Lewis and Lewis (1955) found these two species to be genetically incompatible; no hybrids were formed following interspecific pollination. This incompatibility means that, while divergent flowering times might help sympatric populations of *C. unguiculata* and *C. cylindrica* to avoid interspecific pollination and stigma clogging, phenological divergence is not needed to achieve reproductive isolation.

Both species are pollinated by a small number of specialist, solitary “Clarkia bees” (Lewis and Lewis 1955; MacSwain et al. 1973) that are attracted to the conspicuously pigmented, flecked, and spotted cup-shaped flowers of *C. cylindrica* and the rotate, clawed-petalled flowers of *C. unguiculata*, both of which provide pollen and nectar rewards (Fig. 1). *Clarkia cylindrica* is commonly visited for its pollen by *Andrena lewisorum* Thorp, which has also been found to visit *C. unguiculata* (MacSwain et al. 1973). Other bee species that visit *C. cylindrica* and *C.*

unguiculata (but not necessarily at the same locations, at similar frequencies per plant species, or with equivalent pollination efficacy) include: *Andrena omninigra* Viereck, *Hesperapis regularis* (Cresson), *Megachile gravata* Mitchell, *M. pascoensis* Mitchell, *Diadasia angusticeps* Timberlake, *Melissodes clarkiae* LaBerge, *Synhalonia venusta carinata* (Timberlake), *Ceratina sequoiae* Michener, *Lasioglossum pullilabre* (Vachal) and *Apis mellifera* Linnaeus (MacSwain et al. 1973).

Herbarium Data

The two *Clarkia* species examined here are well represented in the holdings of the Consortium of California Herbaria. For the current study, we borrowed specimens of each species from the Jepson Herbarium (JEPS) and the University Herbarium (UC) at the University of California, Berkeley; Rancho Santa Ana Botanic Garden (RSA); the University of California, Riverside (UCR), and the Santa Barbara Botanic Garden (SBBG). Each specimen’s label was examined to extract its collection date (recorded as the day of year on which the specimen was collected [1–365, or 366 on leap years], year of collection, elevation (m), latitude and longitude. Specimens that lacked GPS coordinates were georeferenced using the label’s description of the collection site and the online utility, GEOLOCATE (<http://www.museum.tulane.edu/>

geolocate/). Where elevation was not recorded on the label, we used the specimen's latitude and longitude (with GEOLOCATE) to estimate its elevation. Only specimens that bore one or more individual plants that had begun to reproduce (i.e., bearing flower buds, open or spent flowers, expanding ovaries, or fruits) at the time of collection were included in this study. Individual plants that had not begun to reproduce were not scored.

Because herbarium specimens may have been collected at any time during an individual's reproductive cycle, a specimens' collection date (DOY) is not a precise proxy for date of onset of flowering. Moreover, DOY is generally positively correlated with an individual plant's phenological status such that, under similar environmental conditions, individuals collected at early stages of reproduction (e.g., when bearing only closed flower buds) are represented by earlier DOYs than those collected at later stages (e.g., when bearing only ripe fruits). Because DOY is confounded with reproductive stage, predictive models that control for the phenological status of sampled plants when examining the relationship between DOY and climatic conditions explain a higher proportion of the variance in DOY than those that do not control for this source of variance (Love et al. 2019).

To provide a quantitative estimate of each specimen's phenological status, we used a phenological index (PI) that ranges from 1 (for plants comprised entirely of buds) to 4 (for plants comprised of all fruits) (Love et al. 2019). For each individual plant specimen (including multiple plants when a given herbarium sheet contained more than one individual), we counted the numbers of buds (>5 mm in length), open flowers, wilted flowers or expanding ovaries, and fully developed fruits (full-sized and/or beginning to dehisce). Each organ type was assigned a value that reflected its developmental stage (buds = 1; open flowers = 2; spent flowers or expanding ovaries = 3; fully developed fruits = 4) and used in the following equation:

$$\text{Phenological index (PI)} = \sum_{i=1}^4 ((P_x)(i))$$

where P_x represents the proportion of reproductive organs in a given stage and i represents the value assigned to that class (e.g., buds have a value of 1). The PI is therefore the weighted average of the proportions of buds, open flowers, spent flowers or developing ovaries, and ripe fruits. For herbarium sheets on which multiple, complete individuals were pressed, we assigned the sheet the mean of the PI values of its component individuals. Partial individuals were not scored for their PI. For *C. unguiculata*, a total of 608 plants on 231 sheets were scored; for *C. cylindrica*, a total of 1042 plants on 306 sheets were scored.

Duplicate specimens were considered to be those that were: collected <500 m away from the nearest

specimen in both latitude and longitude; collected on the same day of the same year; and represented by the same mean annual temperature normal, mean spring maximum temperature normal, and annual precipitation normal (as extracted using the climate database, ClimateNA; Wang et al. 2016). Two sites separated by latitudinal and longitudinal distances of 500 m would be a linear distance of ~ 707 m apart, and were usually associated with distinct climate variables due to a difference in slope, aspect, or elevation, which ClimateNA uses to estimate climatic parameters. Following the elimination of duplicate specimens, 226 sheets of *C. unguiculata* collected from 1902–2011 and 284 sheets of *C. cylindrica* collected from 1900–2012 were analyzed here.

Within each species, some specimens were retained even if they were collected <707 m from another collection site. For 85 specimens of *Clarkia cylindrica*, the distance to the nearest collection site was <707 m, but only 24 of these specimens (distributed in 10 groups of 2 or 3 specimens) were collected on the same day in the same year as a nearby specimen. Based on the data retrieved from ClimateNA, the 2–3 specimens in each of these groups were represented by different combinations of mean annual temperature, annual precipitation, and mean Spring Tmax (likely due to differences between them in slope, aspect, and/or elevation, for which ClimateNA interpolated distinct climate parameters), and so they were retained for analysis. Twenty-two of the 226 specimens of *Clarkia unguiculata* were collected <707 m from the next closest site. Of these specimens, two were collected on the same day in the same year as their nearest neighbor, but 40 meters apart in elevation.

The range of PI values recorded for the sheets of *C. cylindrica* was 1.0–3.77; the range for *C. unguiculata* was 1.0–3.95. In all analyses described below, the PI was log-transformed to improve normality.

Climate Data

We evaluated climate variables commonly found to influence flowering date in other taxa (Anderson et al. 2012; Cleland et al. 2012; Park and Mazer 2018; Berg et al. 2019): annual precipitation (AP; this includes cumulative rain and snow, with the latter converted to water-equivalents); mean maximum spring temperature (Spring Tmax, the mean maximum daily temperature from March–April), mean minimum spring temperature (Spring Tmin), and the number of frost-free days in winter and in spring (NFFD). For each collection site and climatic parameter, two values were extracted from ClimateNA (Wang et al. 2016), an application that assembles downscaled monthly climatic parameters (e.g., the mean of daily values) for a wide range of parameters recorded from 1901 onwards. First, we obtained the long-term mean value of each parameter from 1921–2010. Second, we extracted the climate conditions for the year of collection (YOC). For each collection site

× year combination, we then calculated the deviation between the annual conditions in the YOC and long-term climate mean (hereafter referred to as the “normal”). The value of this deviation (referred to here as the “anomaly” for the focal variable) indicates whether, in the year of specimen collection, a given site was warmer- (or cooler) or drier- (or wetter) than its long-term average.

Exploratory linear models were constructed and tested to determine whether Spring Tmax or Spring Tmin better explained variation in DOY. Each of two models was tested separately on *C. cylindrica* and *C. unguiculata*, and then on the pooled data including both species. The first model included the following variables as predictors: Log(PI), AP normal, Spring Tmax normal, AP anomaly, and Spring Tmax anomaly. The second included the same predictors but using the Spring Tmin normals and anomalies instead of Tmax. The models in which the predictor variables included Spring Tmax performed better (had higher R^2 values) than those that included Spring Tmin (Appendix S1). Because Spring Tmax and Spring Tmin are collinear among collection sites (Spring Tmax normal vs. Spring Tmin normal: $r = 0.55$, $P < 0.0001$, $n = 509$; Spring Tmax anomaly vs. Spring Tmin anomaly: $r = 0.75$, $P < 0.0001$, $n = 509$), we did not include both in the same model.

In addition to examining the effects of Annual Precipitation, Spring Tmax, and Spring Tmin on flowering date, we used data from ClimateNA to calculate the anomalies for Winter Tmax, Winter Tmin, precipitation as snow (PAS), and the number of frost-free days (NFFD) in winter and spring in order to examine long-term temporal trends in climate over the ~115 yr of specimen collection represented by the data analyzed here.

Analyses

Climatic conditions and geographic locations occupied by each species. To compare species with respect to the combinations of climatic and geographic conditions they occupy throughout their sampled ranges, we examined the bivariate space occupied by each species' collection sites with respect to their Spring Tmax and AP normals, as well as their elevation, latitude, and longitude. Linear models were then constructed to test for significant differences between species' means with respect to each of the four focal climate variables (AP normal, Spring Tmax normal, AP anomalies, and Spring Tmax anomalies) while controlling for variation in the other three variables. In each of these models, the focal climate variable was the response variable and the other three climate variables and Species were included as fixed main effects. One-way ANOVAs were conducted to compare species' means with respect to elevation.

Climate change during sampling period: Temporal change in climate anomalies. Analyzing each species separately over its ~115-yr sampling period, we conducted simple regressions to test for temporal trends in each of our focal climate variables: AP, PAS, winter Tmin, Spring Tmin, Winter Tmax, Spring Tmax, Winter NFFD, and Spring NFFD. In each regression, the anomaly for a given climate variable was included as the response variable and Year as the independent variable. In these analyses, positive slopes of the regression of the temperature- (or precipitation-) based anomalies on year mean that, as time progresses, the sampled sites are becoming warmer (or wetter) than average.

Factors influencing flowering date: species identity, spring Tmax and AP normals and anomalies, and phenological status. We constructed and tested a suite of linear models to detect significant differences between species in mean DOY and to measure the independent effects on DOY of the normals for AP and spring Tmax, of the AP and spring Tmax anomalies, and of the phenological status (estimated as $\log[PI]$) of individuals. Because interactions between climate variables can influence the interpretation of their individual effects on DOY, we also sought evidence for significant interactions between each pair of the climate variables that were included as main effects. This analysis was conducted in several steps; in all models, DOY was the response variable.

The first model tested only for the following main fixed effects: Species, Log(PI), AP and spring Tmax normals, and AP and spring Tmax anomalies. We then tested each of 15 two-way interactions by adding one of the following interactions to the first model to test its contribution to variance in DOY: AP normal × Spring Tmax normal, AP normal × AP anomaly, AP normal × Spring Tmax anomaly, Spring Tmax normal × AP anomaly, Spring Tmax normal × Spring Tmax anomaly, AP anomaly × Spring Tmax anomaly, LogPI × Species, AP normal × Species, Spring Tmax normal × Species, AP anomaly × Species, Spring Tmax anomaly × Species, AP normal × Log(PI), Spring Tmax normal × Log(PI), AP anomaly × Log(PI), and Spring Tmax anomaly × Log(PI). We chose to test the two-way interactions individually rather than to include all of them in a single model in order to facilitate the biological interpretation of the coefficients of each interaction and main effect. Additionally, potential collinearity between predictors (such as the cross-products that comprise interactions) can lead to variance inflation, making detection of significant effects difficult (i.e., increased Type II errors) when models contain all possible predictors.

Among all of these models, the only significant two-way interaction was the AP normal × Tmax normal interaction (see Results). This interaction term was also statistically significant ($P = 0.0178$) when tested in a model that included the six main

effects above plus all of the two-, three-, and four-way interaction terms between and among the four climate variables (AP and Spring Tmax normal and anomalies; results not shown). We then tested for differences between species with respect to this interaction by constructing and testing a linear model that included the following predictors: Species, Log(PI), AP and Spring Tmax normals, AP and Spring Tmax anomalies, and the AP normal \times Tmax normal and the Species \times MAP normal \times Tmax normal interaction terms.

Long-term phenological shifts. To detect long-term phenological shifts in flowering dates in each species across the sampling period, we constructed and tested linear models in which DOY was the response variable and log(PI), year, latitude, longitude, and elevation were treated as fixed independent variables. In these main-effects models, there was no significant effect of year on DOY. We then tested for two-way interactions between year and each geographic attribute: Year \times Latitude, Year \times Longitude, and Year \times Elevation. Each two-way interaction was tested separately by adding it to the main-effects model. In the absence of any significant two-way interaction, a significant (or non-significant) effect of Year on DOY could be interpreted as an effect of Year that is independent of the effects on DOY of latitude, longitude, or elevation. In turn, a significant interaction would indicate that the rate of change in DOY over time (i.e., the effect of Year) differs among specimens collected in different latitudes, longitudes, or elevations.

All linear models were conducted using the *lm* function (stats 4.0.2 of the R Stats Package) in R Studio version 1.2.5042; figures were created using *visreg* v2.7.0 (Breheny and Burchett 2017), and *ggplot2* v3.3.2 (Wickham 2016). In all of these linear models, significance testing was conducted using Type III sums of squares; the effects on DOY of each independent variable or interaction term was tested when placed last into the model. In multiple linear models that included species as an independent variable, the *lsmeans* and *eff_size* functions (in the *emmeans* package) were used to test for significant differences between species' least squares means and for effect sizes.

RESULTS

Climatic Conditions and Geographic Locations Occupied by Each Species

Clarkia cylindrica and *C. unguiculata* are endemic to California, with *C. unguiculata* being the more geographically widespread of the two (Fig. 1). Bivariate plots illustrating the joint distributions of the sampled specimens of the two species with respect AP normal, spring Tmax normals, elevation, longitude and latitude reveal broad overlap between the two species with respect to all variables, although *C.*

unguiculata extends farther to the north, west, and southeast of California than *C. cylindrica* (Fig. S1).

The linear models designed to test for differences between the two species with respect to their exposure to long-term variation in climate detected that the specimens of *C. cylindrica* were collected from chronically drier and cooler sites than those of *C. unguiculata* (Fig. 2). The least squares mean (LSM) for the AP normal from 1921–2010 was 463 mm (\pm SE = 10.3) for *C. cylindrica* and 563 mm (\pm SE = 11.5) for *C. unguiculata* (effect size = $0.58 \pm$ SE = 0.09, df = 504, $P < 0.0001$). The LSM for the Spring Tmax normal was 19.8°C (\pm SE = 0.107) for *C. cylindrica* and 20.4°C (\pm SE = 0.120) for *C. unguiculata* (effect size = $0.321 \pm$ 0.92, df = 504, $P = 0.0005$). The collection sites of the two species, however, exhibit similar and near-zero mean values of the AP and spring Tmax anomalies (Fig. 2), indicating that neither species was disproportionately represented by sites that were hotter (or cooler) or drier (or wetter) than average in the year of collection.

The mean elevation of the sites from which *C. cylindrica* was collected was significantly higher than that of the sites from which *C. unguiculata* was sampled (one-way ANOVA of log-transformed values of elevation: $F_{1, 507} = 25.95$, $P < 0.0001$, $R^2 = 0.05$). *Clarkia cylindrica* specimens were sampled at a mean elevation of 636.3 m (\pm SE = 21.90, $n = 283$) and *C. unguiculata* specimens were collected at a mean elevation of 493.4 m (\pm SE = 23.4, $n = 226$) (Fig. S2).

Climate Change During Sampling Period: Temporal Change in Climate Anomalies

Both species were exposed to similar patterns of temporal change in climate anomalies over the ~115-yr sampling periods with respect to AP anomalies, winter and spring Tmax and Tmin anomalies, and the anomalies for the number of frost-free days (NFFD) in winter and spring. Both species experienced small, but statistically significant, increases in AP anomalies (90.4 and 114 mm/100 yrs, respectively for *C. cylindrica* and *C. unguiculata*) (Table 1). Similarly, both species experienced significant temporal increases in winter and spring Tmin anomalies (Fig. 3), but no significant change in either winter or spring Tmax anomalies over the sampling period. Finally, both species experienced similar and statistically significant temporal increases in the number of frost-free days in both winter and spring (Fig. 3).

Factors Influencing Flowering Date: Species, Spring Tmax, and AP Normals and Anomalies, and Phenological Status

Differences between species' mean DOY. Controlling for the effects on DOY of the other predictor variables in the model, *C. cylindrica* flowers 3.46 d earlier (\pm SE = 1.55; effect size = $-0.21 \pm$ SE = 0.932, df = 501) than *C. unguiculata* (LSM of DOY for *C.*

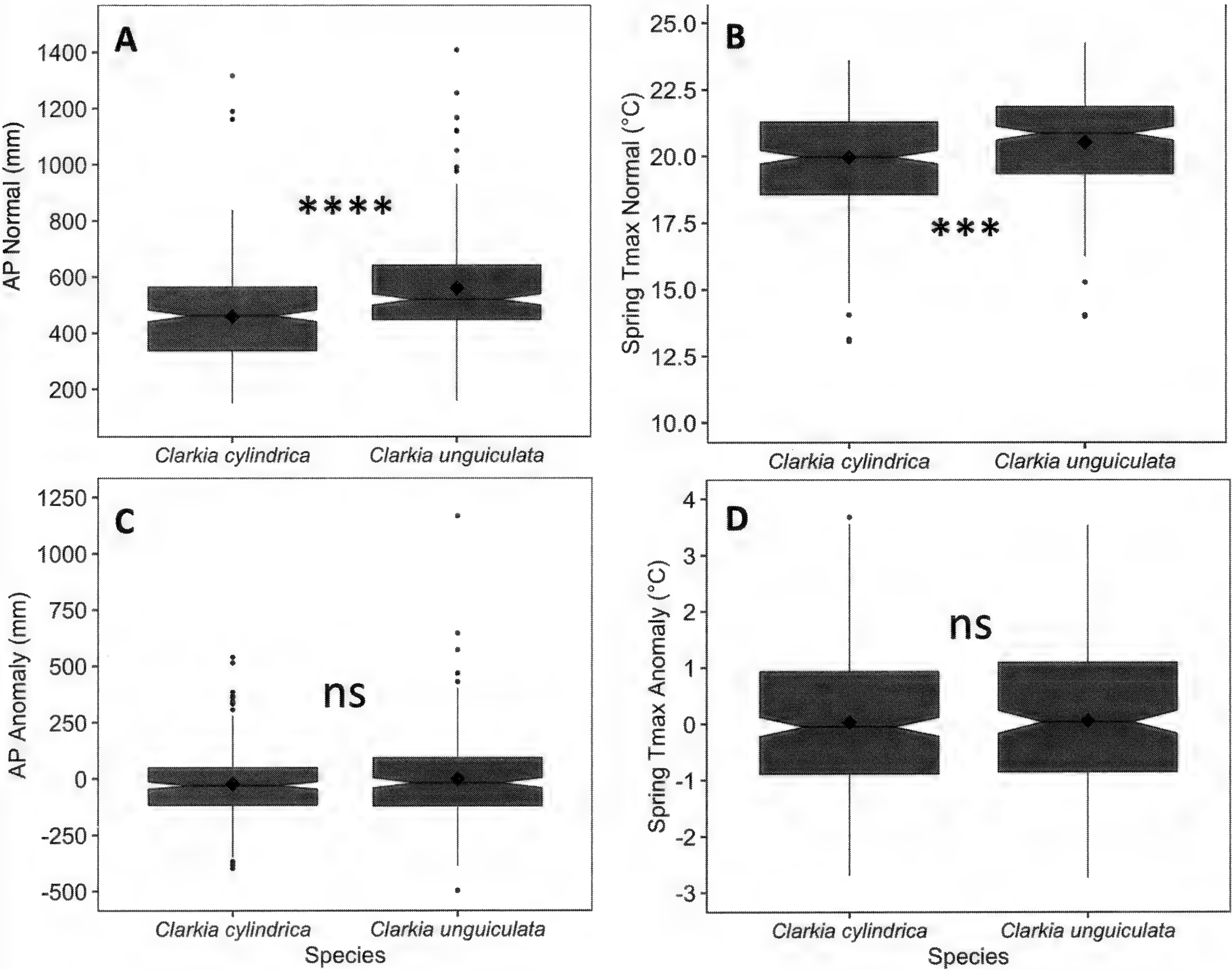


FIG. 2. Box plots displaying the least squares means (LSMs) for *C. cylindrica* and *C. unguiculata* for each of four climatic variables, where the LSM of the focal dependent variable for each species was estimated while controlling for variation in the other three variables. Based on the specimens sampled here, the two species differ significantly with respect to the mean values of the chronic climatic conditions (mean annual values from 1921–2010) for cumulative annual precipitation (AP) and Spring Tmax. *C. cylindrica* occupies drier and cooler sites than *C. unguiculata*. Horizontal black lines represent the median of each climatic variable; diamonds represent the mean values. Within each panel, if the outer openings of the notches of the two species do not overlap, this can be interpreted as a significant difference between the median values of the two species.

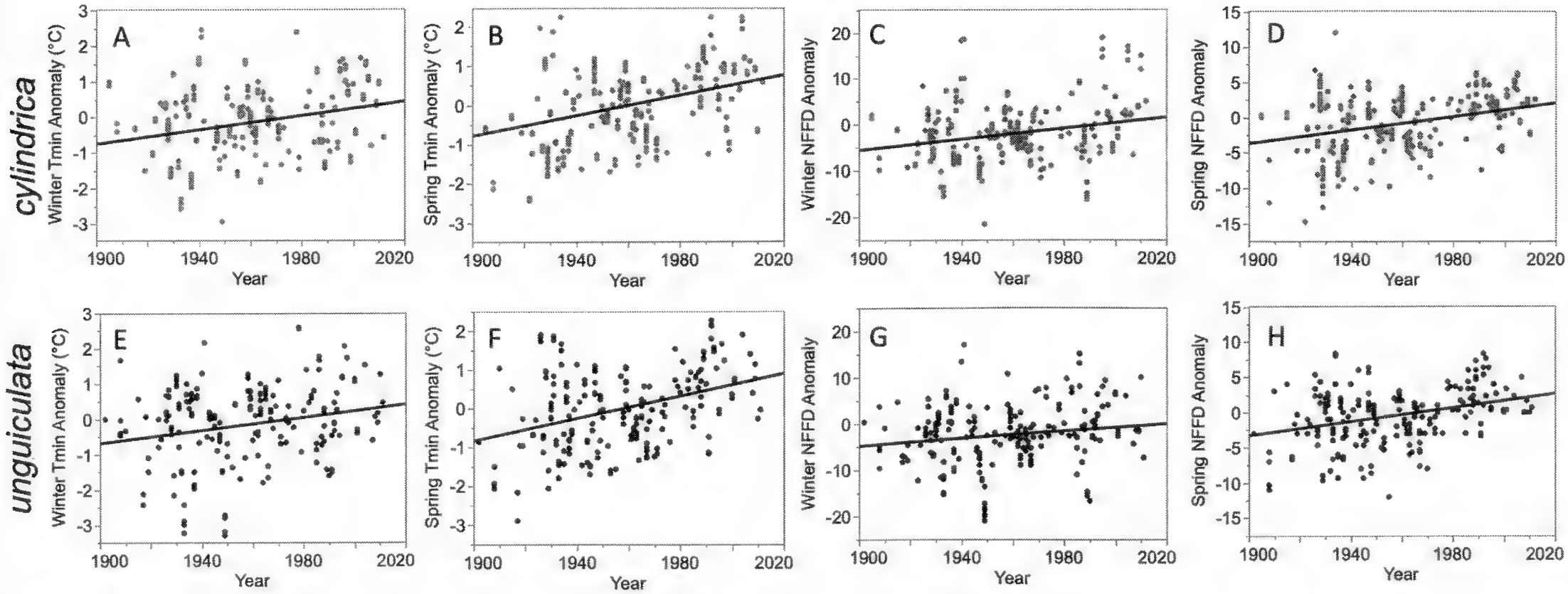


FIG. 3. Bivariate regressions illustrating significant temporal trends in winter and spring Tmin anomalies (the deviations between the climate normals and conditions in the year of specimen collection at each site) and in the anomalies for the number of frost-free days (NNFD) in winter and spring. See Table 1 for regression parameters.

TABLE 1. SUMMARY OF BIVARIATE REGRESSIONS CONDUCTED USING THE SPECIMENS OF EACH SPECIES SEPARATELY TO DETECT TEMPORAL TRENDS IN CLIMATE ANOMALIES OVER THE ~115-YEAR SAMPLING PERIOD. The two species show similar magnitudes of temporal change in the anomalies for cumulative annual precipitation (AP, including rain and snow), PAS (precipitation as snow), winter and spring mean minimum daily temperature (Tmin), and winter and spring NFFD (number of frost-free days), indicating rain significant increases in precipitation and winter and spring temperatures. Boldfaced values indicate variables for which significant change ($\alpha = 0.05$) was detected over the sampling period (values are italicized when $0.05 < P < 0.10$). The inclusion of elevation, latitude, and longitude as main effects in these models did not significantly change the magnitude of the parameter estimate for the effect of year on any climate variable.

Climate variable	<i>C. cylindrica</i> (n = 283)				<i>C. unguiculata</i> (n = 226)			
	Slope	SE	P-value	R ² (Adjusted)	Slope	SE	P-value	R ² (Adjusted)
AP	0.904 (90.4 mm/100 yr)	0.380	0.0188	0.016	1.14 (114 mm/100 yr)	0.53	0.0320	0.016
PAS	-0.0035 (-3.5 mm/100 yr)	0.030	ns	0.00	-0.195 (-1.95 mm/100 yr)	0.05	ns	0.00
Winter Tmin	0.010 (1.00°C/100 yr)	0.002	< 0.0001	0.07	0.009 (0.9°C/100 yr)	0.003	0.0026	0.04
Spring Tmin	0.013 (1.27°C/100 yr)	0.002	< 0.0001	0.12	0.015 (1.5°C/100 yr)	0.003	< 0.0001	0.12
Winter Tmax	0.004 (0.4°C/100 yr)	0.002	ns	0.01	0.006 (0.6°C/100 yr)	0.003	0.0686	0.01
Spring Tmax	-0.0002 (0.02°C/100 yr)	0.003	ns	0.00	0.004 (0.4°C/100 yr)	0.004	ns	0.00
Winter NFFD	0.060 (6.0 d/100 yr)	0.014	< 0.0001	0.06	0.040 (4.0 d/100 yr)	0.018	0.0221	0.02
Spring NFFD	0.049 (4.9 d/100 yr)	0.009	< 0.0001	0.09	0.050 (5.0 d/100 yr)	0.010	< 0.0001	0.10

cylindrica = 146.0 ± SE = 1.03; LSM for *C. unguiculata* = 149.0 ± SE = 1.15; t-ratio = 2.232, P = 0.0260) (Table 2; Fig. S3).

Effect of long-term, chronic climatic conditions. The linear model applied to the data set in which the two species were pooled detected significant effects of AP normals and Spring Tmax normals on DOY while controlling for the phenological status of specimens, inter-annual variation in AP and spring Tmax, and the two-way interaction between AP normals and spring Tmax normals (Table 2).

The significant two-way interaction between AP and Spring Tmax normals indicates that the delaying effects of high precipitation are stronger in warmer than in cooler regions and that the advancing effects of higher spring Tmax are stronger in chronically dry than in relatively wet conditions (Fig. 4). Flowering consistently occurs earliest under hot and dry conditions. This interaction did not differ significantly between species (Appendix S2; the Species × AP normal × Spring Tmax normal interaction was non-significant) although when we tested species-specific models, a significant interaction was detected in *C. cylindrica* but not in *C. unguiculata* (Fig. S4, Appendix S3). We detected no evidence in the analysis of the pooled data set that species differed in their sensitivity to AP or Spring Tmax normals. When added to the model containing only main effects (Species, PI, AP normals and anomalies, and Spring Tmax normals and anomalies), no significant two-way interactions were detected between Species and either AP or Spring Tmax normals (results not shown).

Effects of inter-annual variation in AP and Spring Tmax. Among all specimens, we detected a 1.0-d delay for every 100 mm increase in the AP anomaly and a 4.2-d advancement for every 1°C increase in the Spring Tmax anomaly. We detected no significant two-way interaction between the AP normal and Spring Tmax anomalies (Table 2). Moreover, we detected no evidence in the analysis of the pooled data set that species differed in their sensitivity to AP or Spring Tmax anomalies. When added to the model containing only main effects, no significant two-way interactions were detected between Species and either AP or Spring Tmax anomalies (results not shown).

Effects of phenological status on flowering date. When added to the model containing only main effects, we detected no significant PI × Species interaction, indicating that the rate of phenological progression over time is the same for both species (Fig. 5). The necessarily positive slope of the relationship between DOY and log(PI) (i.e., the regression coefficient associated with log[PI] in Table 2) indicates how rapidly plants progress from bearing all buds to bearing only fruits. Higher values of this slope indicate that it takes longer to progress between phenological stages; lower values (flatter lines)

TABLE 2. SUMMARY OF MEAN-CENTERED LINEAR MODEL DESIGNED TO DETECT SIGNIFICANT EFFECTS ON THE DAY OF YEAR (DOY) OF SPECIMEN COLLECTION OF PHENOLOGICAL STATUS, SPECIES, GEOGRAPHIC AND INTER-ANNUAL VARIATION IN CLIMATE, AND THE TWO-WAY INTERACTION BETWEEN LONG-TERM ANNUAL PRECIPITATION (AP NORMAL) AND LONG-TERM SPRING TMAX (MEAN MAXIMUM DAILY TEMPERATURE). The values of the phenological index were log-transformed to improve normality. No significant interactions were detected between Species \times AP normal, Species \times Spring Tmax normal, AP normal \times Spring Tmax anomaly, or Species \times AP normal \times Spring Tmax normal, so these interactions are excluded here. The effects on DOY of the main effects and interactions shown here do not differ between species. Type III sums of squares were used for significance testing.

Analysis of variance	df	Sum of squares	<i>F</i>	P-value
Source				
Intercept	1	10032046	36817	<0.0001
Phenological Index	1	32314	116.56	<0.0001
Species	1	1381	4.98	0.026
AP normal	1	4531	16.35	<0.0001
Spring Tmax normal	1	12036	43.41	< 0.0001
AP anomaly	1	1663	6.00	0.015
Spring Tmax anomalies	1	13573	48.96	<0.0001
AP normal \times Spring Tmax normal	1	1636	5.90	0.015
Residuals	501	138893		
Model Adjusted $R^2 = 0.34$			38.41	<0.0001
Parameter estimates				
	Estimate	SE	t-value	P-value
Intercept	147.27	0.774	190.23	<0.0001
Phenological Index	67.98	6.296	10.80	<0.0001
Species (<i>C. cylindrica</i>)	−1.73	0.774	−2.23	0.026
AP normal	0.018	0.005	4.04	<0.0001
Spring Tmax normal	−4.18	0.418	−6.59	<0.0001
AP anomaly	0.010	0.004	2.45	0.015
Spring Tmax anomaly	−4.19	0.598	−7.00	<0.0001
AP normal \times Spring Tmax normal	0.003	0.001	2.43	0.016

indicate that progression between stages occurs more rapidly.

Just as the rate of phenological progression did not differ between species, the rate of progression was also unaffected by the climatic variables examined here. None of the following two-way interactions were significant: PI \times AP normal, PI \times Spring Tmax normal, PI \times AP anomaly, or PI \times Spring Tmax anomaly (results not shown), indicating that the rate of phenological progression did not depend on local climatic conditions.

Long-term Phenological Shifts

Neither species exhibited statistically significant phenological change over the ~115-yr sampling period, independent of the effects on DOY of phenological status, elevation, latitude and longitude (Table 3). These models detected, however, that in both species DOY changes significantly with elevation and longitude; higher elevations and more western locations are associated with delayed flowering (Fig. 6). The species-specific models containing only main effects (Table 3) have the same adjusted model R^2 values (0.23 for *C.*

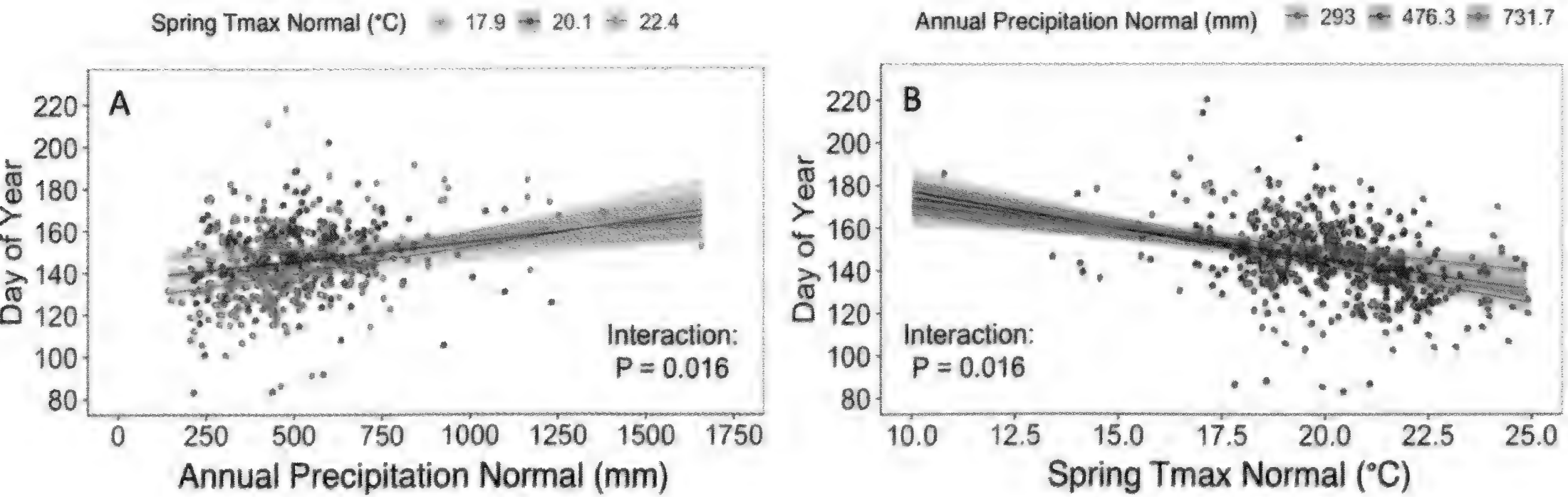


FIG. 4. Illustration of the significant interaction between long-term mean annual precipitation (Annual Precipitation Normal) and long-term mean maximum spring temperature (Spring Tmax normal) detected in the analysis of pooled data (Table 2). A. The delaying effects on DOY of high precipitation are strongest in relatively warm regions. B. The advancing effects on DOY of higher mean Spring Tmax are strongest in relatively dry regions.

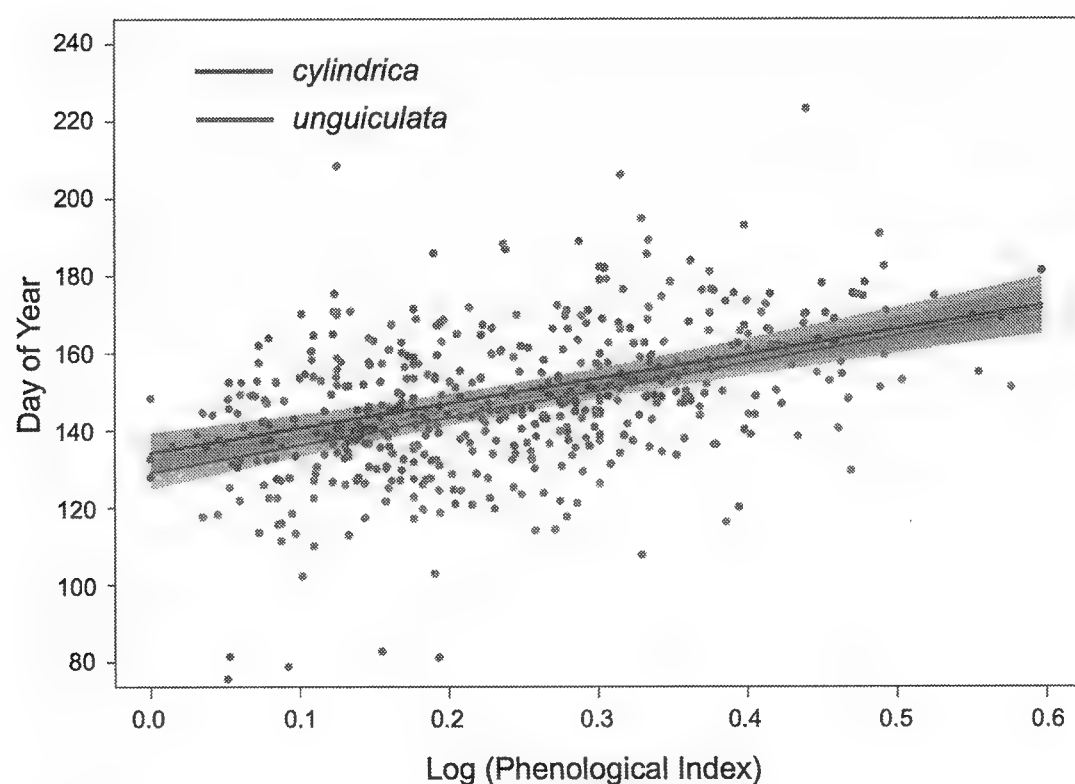


FIG. 5. Leverage plots illustrating the relationship between day of year of specimen collection and the phenological status of herbarium specimens representing *Clarkia cylindrica* and *C. unguiculata*. Herbarium specimens represented by a value of 0 for the Log(Phenological Index) bear only buds; those represented by a value of 0.6 bear only ripe fruits. The absence of a significant Species \times Log(PI) interaction (Table S5) indicates that the slope of the relationship shown here ($67.29 \pm \text{SE} = 6.42$) does not differ between species.

cylindrica; 0.19 for *C. unguiculata*) as the three species-specific models that each contained a two-way interaction (Year \times Elevation, Year \times Latitude, or Year \times Longitude), none of which were significant (Table S4: Adjusted R^2 values for models including the Year \times Elevation, Year \times Latitude, and Year \times Longitude interaction term, respectively, are 0.23, 0.23, and 0.24 for *C. cylindrica*, and 0.19, 0.19 and 0.19 for *C. unguiculata*).

DISCUSSION

Among the specimens sampled here, *C. cylindrica* and *C. unguiculata* exhibit more similarities than differences in their phenological behavior. They do not differ in their sensitivities to chronic local climatic conditions (normals) or to year-specific conditions (anomalies). In addition, they exhibit the same rates of progression through successive phenological stages (Fig. 5), which appear to be constant throughout their ranges (there were no significant interactions between PI and any of the four focal climate variables). If these similarities are in fact the result of adaptive evolution, then the benefits of co-flowering would appear to have outweighed the benefits of divergence and reproductive isolation. These similarities predict that, all else being equal, as both precipitation and spring temperature change in the future, the two species' flowering phenology should shift to the same degree and in the same direction.

Difference Between Species in the Timing of Flowering

The specimens of *C. cylindrica* and *C. unguiculata* sampled here differ with respect to the historical

conditions at the sites from which they were collected, with *C. cylindrica* occupying drier and cooler locations than *C. unguiculata* (Fig. 2). This difference between the species in the mean climatic conditions of the sampled sites from 1920–2010, however, does not explain the statistically significant 3.5-d difference between them in DOY, because independent of local climatic conditions (both the normals and the anomalies) and the phenological status of specimens, this 3.5-d difference persists (Table 2). Whether this difference between species in mean DOY represents the adaptive outcome of natural selection due to species-specific effects of flowering time on pollination, herbivory, or the intensity of inter-specific competition, or whether it represents an evolutionary or plastic response to other environmental differences between the species that were not investigated here, cannot be determined without additional research. More fundamentally, it is not clear that a difference of this magnitude has any ecological or fitness consequences.

Species Exhibit Similar Sensitivities of Flowering Time to Local Temperature and Precipitation, and Similar Rates of Phenological Progression

The absence of statistically significant interactions between species and the focal climate variables (Species \times AP normal, Species \times Spring Tmax normal, Species \times AP anomaly, Species \times Spring Tmax anomaly, and Species \times MAP normal \times Spring Tmax normal; results not shown) indicates that species do not differ with respect to the direction or magnitude of their responses to local AP or Spring Tmax normals or to warmer- or wetter-than average conditions in the year of collection (represented by the AP and Spring Tmax anomalies). In addition, the lack of a significant Species \times Log(PI) interaction (Table S5) and the parallel leverage plots of the relationship between DOY and log(PI) (Fig. 5) indicate that the rates at which the two species progress through their successive phenological stages do not differ, based on these analyses.

Other studies of congeners have observed both similarities and differences in their phenological behavior, in part depending on whether the species' native ranges encompassed the same climatic conditions. In a study of four *Quercus* species — two species restricted to a water-limited, Mediterranean climate in California (*Q. agrifolia* Née and *Q. lobata* Née) and two species occupying the temperate zone of eastern and central North America (*Q. alba* L. and *Q. rubra* L.) — Gerst et al. (2017) found that individual trees of the western species tended to flower intermittently throughout the growing season and for much longer durations than the eastern/central species, which exhibited much more synchronous and fewer flowering onset dates. Gerst et al. (2017) interpreted the western species' behavior as a potential adaptive evolutionary or plastic response to unpredictable episodes of rainfall throughout the late

TABLE 3. SUMMARY OF SPECIES-SPECIFIC PARAMETER ESTIMATES (SLOPES) FOR MEAN-CENTERED LINEAR MODELS DESIGNED TO DETECT LONG-TERM TEMPORAL TRENDS IN FLOWERING DATE (DOY) WHILE CONTROLLING FOR VARIATION IN PHENOLOGICAL STATUS (LOG[PI]), ELEVATION, LATITUDE, AND LONGITUDE. A. *Clarkia cylindrica*. B. *C. unguiculata*. Two-way interactions involving year (Year \times Elevation, Year \times Latitude, and Year \times Longitude) were all non-significant (see Table S4), supporting the interpretation that the effects of year on the day of year of specimen collection are consistent across elevation, latitude, and longitude. Type III sums of squares were used for significance testing.

A. *Clarkia cylindrica*

Analysis of variance	df	Sum of squares	<i>F</i>	P-value
Source				
Intercept	1	5940689	20005	<0.0001
Phenological Index	1	20433	68.81	<0.0001
Year	1	370	1.25	0.27
Elevation	1	7070	23.81	<0.0001
Latitude	1	708	2.38	0.12
Longitude	1	998	3.36	0.07
Residuals	278	82555		
Model Adjusted R ² = 0.23			18.18	<0.0001
Parameter estimates	Estimate	SE	t-value	P-value
Intercept	144.63	1.023	141.44	<0.0001
Phenological Index	69.73	8.406	8.30	<0.0001
Year	−0.043	0.039	−1.12	0.27
Elevation	0.016	0.003	4.88	<0.0001
Latitude	−1.91	1.236	−1.54	0.12
Longitude	−2.75	1.502	−1.83	0.07

B. *Clarkia unguiculata*

Analysis of variance	df	Sum of squares	<i>F</i>	P-value
Source				
Intercept	1	5021450	13710	<0.0001
Phenological Index	1	7549	20.61	<0.0001
Year	1	448	1.22	0.27
Elevation	1	9883	26.98	<0.0001
Latitude	1	1	0.0038	0.95
Longitude	1	2508	6.85	<0.01
Residuals	220	80578		
Model Adjusted R ² = 0.19			11.71	<0.0001
Parameter estimates	Estimate	SE	t-value	P-value
Intercept	149.06	1.273	117.09	<0.0001
Phenological Index	51.93	11.438	4.54	<0.0001
Year	−0.057	0.052	−1.11	0.27
Elevation	0.021	0.004	5.20	<0.0001
Latitude	−0.08	1.273	−0.06	0.95
Longitude	−4.02	1.534	−2.62	<0.01

winter and spring in California. The California oaks appeared to flower opportunistically in response to these pulses of precipitation, while the eastern/central U.S. species tracked the transition between winter and spring, and flowered comparatively synchronously in response to local winter or spring Tmax.

A similar pattern was found among 11 congeneric pairs of high- vs. low-elevation species of perennial herbs in Switzerland; the elevation of origin determined the sensitivity of phenological traits to local growing conditions (Schmid et al. 2017). Each of the 11 species pairs were grown in both a high- and a low-elevation common garden and a suite of phenological events was recorded. The species native to low-elevations showed much stronger plastic

responses of flowering phenology to the growing conditions of the common gardens than their high-elevation counterparts, suggesting that phenological sensitivity is a trait that may evolve in response to local conditions.

An experimental study of two temperate zone congeners of *Echium* (Boraginaceae) detected similar phenological sensitivity to drought and heat stress between the short-lived perennial, *E. plantagineum* L., a native of Southern Europe and the annual, *E. vulgare* L., native to Northern Europe (Descamps et al. 2020). In both taxa, experimentally induced stress advanced flowering time, but the rate at which the two species produced sequential flowers differed, with the perennial species exhibiting stronger pheno-

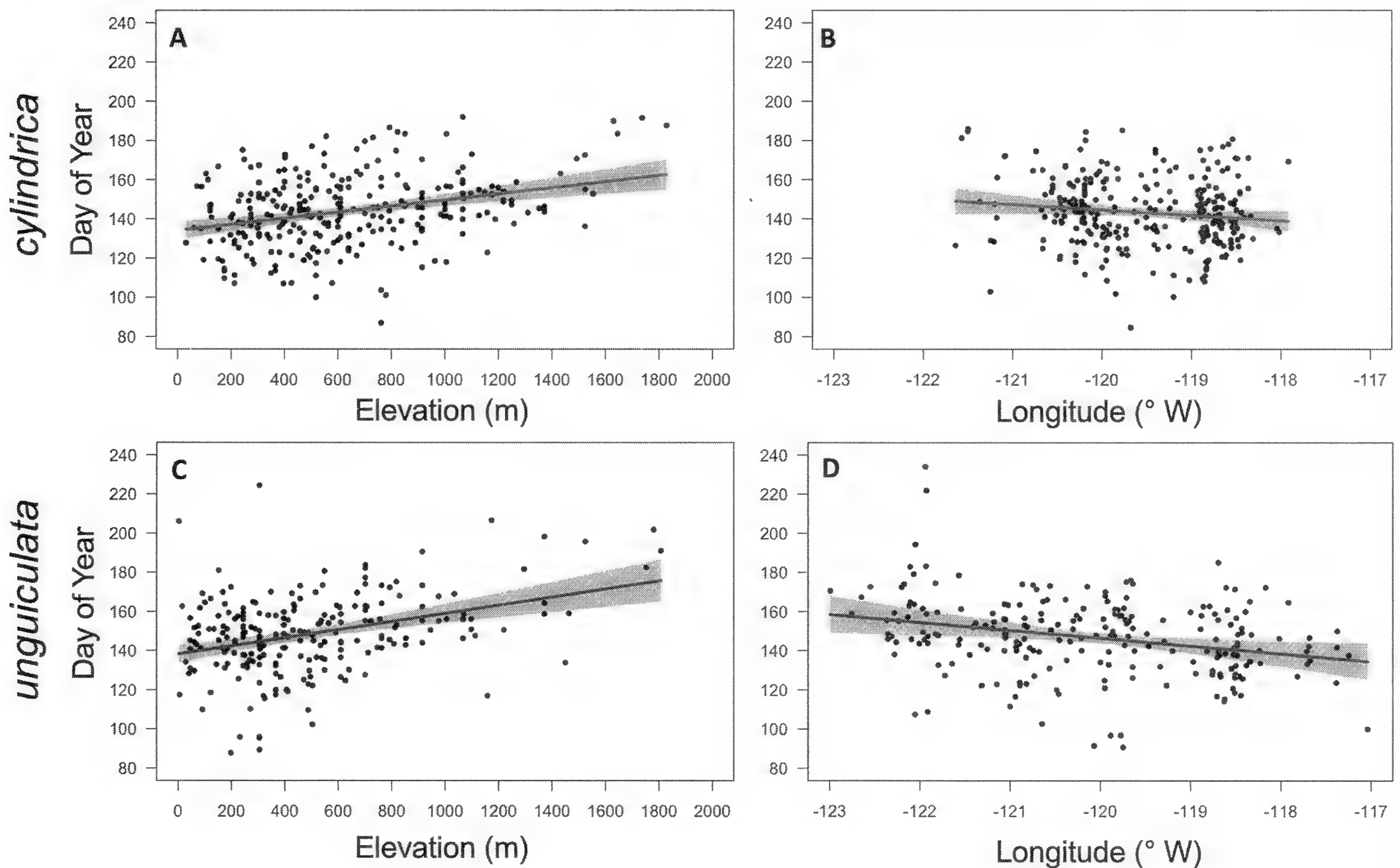


FIG. 6. Leverage plots illustrating the relationship between day of year of specimen collection and elevation (A and C) and longitude (B and D), controlling for variation in the phenological status of specimens, year of collection, and latitude. See Table 3 for parameter estimates (slopes, standard errors, and P-values).

logical responses to the distinct watering and heat treatments than the annual.

Perhaps the most robust way to seek evidence for evolutionary similarities due to the benefits of sharing pollinators (facilitation) vs. the benefits of phenological divergence (reproductive isolation) is to compare the flowering times of congeners when they appear in allopatry vs. sympatry. In a comparative study of 74 pairs of congeners in 26 genera, Park et al. (2020) found that flowering time displacement between congeners observed in sympatry was uncommon, consistent with the hypothesis that the benefits of reproductive isolation are relatively small. Several additional broad, comparative studies have also demonstrated that closely related species tend to have similar flowering dates or sensitivities (Davies et al. 2013 [~4000 species in 1246 genera]; Du et al. 2015 [19631 species in 2284 genera and 181 families], 2020 [2136 species in 846 genera and 158 families]). These studies corroborate the hypothesis that phylogenetic niche conservatism (Wiens and Graham 2005), genetically based trait conservatism (Harvey and Pagel 1991), and parallel or convergent evolution of flowering date (where synchronous flowering facilitates pollinator-sharing) may be more common than selection favoring phenological divergence in flowering time between close relatives. Considered together, these studies suggest that the benefits of co-flowering for pollinator attraction and visitation are greater than the benefits of reproductive isolation.

Sensitivity to Long-Term Climatic Conditions vs. to Inter-Annual Variation in Climate

Higher precipitation and cooler temperatures delay flowering in *C. cylindrica* and *C. unguiculata*, whether those climatic conditions vary geographically or interannually (Table 2). The two species exhibit similar sensitivities to long-term AP normals and AP anomalies ($0.018 \pm \text{SE} = 0.005$ vs. 0.010 ± 0.004 d/mm, respectively) and to long-term spring Tmax normals and anomalies (-2.75 ± 0.42 vs. -4.19 ± 0.60 d/°C, respectively). For these species and climatic variables, therefore, estimates of sensitivity to spatial variation in climate appear to provide reasonable “space-for-time” proxies that can be used to forecast the direction of long-term phenological shifts in response to upcoming change in these parameters. Nevertheless, in the data analyzed here, these sensitivities were not consistent with the absence of statistically significant phenological shifts observed over time.

The linear models tested here include as predictor variables both the climate normals (variation among which represents geographic variation in mean climate conditions) and the climate anomalies (which result from interannual variation in climate) for each site of specimen collection. Accordingly, the effects on DOY of each climatic variable are independent of the effects of the others (Table 2). The significant effects on DOY detected for both the normals and

the anomalies are therefore consistent with the interpretation that both evolutionary adaptation to local temperature and precipitation, and plasticity induced by these parameters in the year of collection, contribute to variation in DOY in these species. In addition, we cannot rule out the possibility that recent, short-term evolutionary responses to temperature and precipitation anomalies also contribute to the associations between DOY and these anomalies (Table 2). Papper and Ackerly (2021) similarly found evidence for both local adaptation and phenotypic plasticity of the phenology of bud break in *Quercus douglasii* Hook. & Arn. (Fagaceae), demonstrating that the ability to distinguish between these processes is not restricted to short-lived annual species.

Current end-of-century climate projections in California predict statewide increases in temperature that range from 2–4°C (assuming an intermediate level of emissions) and from 4–7°C (assuming no mitigation of emissions), while predicted changes in annual precipitation are regional, season-specific, and less certain, as inter-annual variation in precipitation is expected to increase (Pierce et al. 2018). Assuming that future phenological shifts in these taxa can be predicted from observed responses to inter-annual variation in mean spring maximum temperature (i.e., to the anomalies) alone, we would predict that upcoming warming in California will generate phenological advances in their flowering that range from ~8.4–28 d by the end of the century. The phenological response to joint changes in both temperature and precipitation, however, as well as the consequences of these shifts for altering the exposure of wild *Clarkia* populations to herbivores, to pathogens, and to pollinators are obviously unknown.

Long-term Phenological Shifts

Although both species exhibit significant phenological advancement in response to warmer-than-average years (–4.19 d/°C increase in spring Tmax; Table 2), this sensitivity did not generate significant phenological change in either species over the ~115-yr sampling period (Table 3; the effect of year on DOY was not significant). The simplest explanation for this is that there was not a sufficient increase in Spring Tmax over the sampling period in either species to induce detectable shifts in DOY over time (Table 1). However, Spring Tmin anomalies did increase significantly over time (1.27°C/century for *C. cylindrica* and 1.45°C/century for *C. unguiculata*; Table 1), and DOY was sensitive to increases in Spring Tmin anomalies (advancing 3.2 d/°C; Table A6). These values predict an advancement in DOY of 4.1 d/century in *C. cylindrica* and 4.6 d/century in *C. unguiculata*, which were not observed. Other factors that affect DOY and that changed over the sampling period but were not examined here may have opposed this predicted advancement in flowering.

One factor that could have opposed a long-term advancement in DOY is the temporal increase in annual precipitation anomalies (90.4 mm/century for *C. cylindrica* and 114 mm/century for *C. unguiculata*; Table 1). Given the sensitivity of these species to AP anomalies (a delay in DOY of 0.01 d/mm increase in the anomaly; Table 2), the temporal increase in AP over the sampling period is expected to have generated a 0.90-d/century delay in flowering in *C. cylindrica* and a 1.14-d/century delay in flowering in *C. unguiculata*. These predicted delays, however, would not have been sufficient to offset the predicted advancement due to warmer spring temperatures.

In a study of five chaparral species of shrubs in the genera *Arctostaphylos* (Ericaceae) and *Ceanothus* (Rhamnaceae), Parker (2021) similarly detected no significant temporal shift in the flowering phenology even though the mean annual temperature of the collection sites increased significantly across the collection period. By contrast, Pearson et al. (2021) detected a significant advancement in flowering time of *Eschscholzia californica* of 0.6 d per decade across its range, with the magnitude of advancement varying with local long-term mean annual temperature.

The models testing for long-term phenological shifts independent of variation in elevation, latitude, and longitude (Table 3) detect that in both species DOY changes significantly with elevation and longitude; higher elevations and more western locations are associated with delayed flowering (Fig. 6). The non-significant interaction terms between year and each of the geographic variables (Table S4) indicate that the temporal trends in phenology did not depend on elevation, latitude or longitude. Therefore, the non-significant effect of year cannot be attributed to biased sampling of elevations or longitudes over time. In other words, higher elevations or more western sites might have been disproportionately sampled in more recent years, cancelling out the advancing effects of warming on DOY, but this is not the case.

Selection on Flowering Date in *Clarkia*: Empirical Evidence from Other Studies

Several studies of wild populations of *Clarkia* species corroborate the view that sympatric populations benefit from the presence of congeners by attracting pollinators and facilitating each other's pollination. Moeller (2005) observed that, in the southern California endemic, *C. xantiana* A.Gray subspecies *xantiana*, populations growing in sympatry with congeners (including *C. cylindrica* and *C. unguiculata*) with which they share pollinators (the most effective of which he reported to be *Hesperapis regularis*, *Lasioglossum pullilabre*, and *Megachile gravita* or *M. pascoensis*) had visitation rates that were two times higher than those of populations growing without congeners (see also Moeller and Geber 2005). In addition, congeneric populations

growing in sympatry had lower degrees of pollen-limited reproduction than those growing in isolation (Moeller 2004). Moreover, populations of *C. xantiana* subsp. *xantiana* growing in sympatry with other congeners did not differ in their mean flowering time from those growing alone, indicating that there is no evidence of character displacement in this trait in response to inter-specific interactions (including possible competition for pollinators) (Moeller 2004).

One may, with caution, use herbarium specimens representing well-sampled taxa to detect the evolutionary effects on flowering time of growing in sympatry or allopatry with congeners (Park et al. 2020). In the current study, due to both the broadly overlapping ranges of *C. unguiculata* and *C. cylindrica*, and the small number of specimens of each taxon collected from sites that appear to be beyond the range of the other taxon (Fig. 1), it would be risky to use the data analyzed here to reliably identify sites of long-term allopatry vs. sympatry. Moreover, specimen-based data indicate only whether a given species was present at a given location and time, but not if it was absent, making it difficult to assert allopatry in the absence of direct field observations. Nevertheless, herbarium-derived data for well-sampled taxa could be used to identify potential areas of allopatry or sympatry that could then be surveyed in the field to confirm presence or absence of each species. Once these areas are identified, phenological data derived from herbarium specimens could be used to address whether, independent of climate, the mean phenological difference between taxa depends on whether they are in sympatry vs. allopatry.

Other evidence supports the view that competition for pollinators does not select for phenological divergence between sympatric *Clarkia* congeners. Eisen et al. (2019a) conducted a field experiment in which the flowering times and the number of co-occurring *Clarkia* species were manipulated by placing potted, late-flowering plants in natural communities with or without earlier-flowering congeners. They found that seed set of the relatively late-flowering taxon, *C. xantiana* subsp. *xantiana*, depended on local floral density, but that its delayed flowering relative to other congeners could not be attributed to selection favoring reduced interspecific competition for pollinators. Eisen et al. (2019b) also report that the relatively late flowering of *C. xantiana* subsp. *xantiana* is not adaptive; plants had higher seed set when they flowered earlier. They proposed, therefore, that the staggered flowering times among co-occurring *Clarkia* taxa may be due to ecological specialization unrelated to pollination, to developmental constraints associated with other components of their life history (e.g., the need to reach a threshold size before flowering), or to genetically based correlations with other traits that are under strong selection and drive the evolution of species-specific flowering times as a consequence.

If these patterns apply to *Clarkia unguiculata* and *C. cylindrica*, then the phenological similarities

between them reported here may reflect phylogenetic niche conservatism (cf. Truszczyński et al. 2021), trait conservatism, or the outcome of selection on flowering time due to facilitation between co-flowering species that share pollinators. It must be emphasized, however, that although herbarium specimen-based studies can detect phenological patterns over a broader geographic and temporal range than short-term field studies, they are limited in their capacity to identify the mechanisms that contribute to observed phenological divergence or convergence, underscoring the need for complementary studies of natural populations. To detect the factors that independently and directly promote synchronous flowering between congeners, additional observational and experimental work in field populations and communities are needed to measure the costs and benefits associated with synchronous vs. asynchronous flowering between congeners. The continued analysis of large herbarium-based data sets that include congeners, however, hold the promise of detecting those phenological similarities and differences between congeners that merit investigation in the first place.

DATA ACCESSIBILITY

The data sets and R code used in this paper are archived in the Dryad Digital Repository (<https://doi.org/10.25349/D9N026>).

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DO HABITAT SHIFTS ALTER FLOWERING PHENOLOGY OVERLAP IN CLOSE RELATIVES? IMPLICATIONS FOR LOCAL COEXISTENCE

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ABSTRACT

Flowering time in plants is a highly variable trait that influences species' resource use and exchange of pollen with con- and heterospecifics. Levin (2009) suggested that habitat shifts within species might cause plastic shifts in flowering phenology, reducing pollen exchange across habitats. Coupled with divergent selection across habitats, diverged flowering time might thus pave the way towards ecological speciation. Some of these ideas may apply across species as well. If close heterospecific relatives share phylogenetically conserved flowering times and negatively affect each other's fitness, habitat shifts to microallopatry might provide a means for local coexistence by close relatives by reducing resource competition, shared enemies, or negative interactions via pollination. Habitat shifts might also select for diverged flowering time, or cause flowering time divergence, if phylogenetically conserved cues arrive at different times across habitats. Here, we ask if flowering phenology is phylogenetically conserved for 208 species at our coastal field site in northern California, whether flowering phenology differs systematically across habitat types, and whether habitat shifts are associated with phenological separation, especially in congeners. Because annuality and perenniality have been shown to be associated with habitat traits and flowering time, we included life history in our analyses as well. We also explore the frequency of habitat shifts between congener and noncongener pairs. We use both field observations and data from Jepson eFlora/Jepson Manual 2 (Baldwin et al. 2012) to explore patterns in flowering phenology. The two data sources were well-correlated across 59 species. Phylogeny, habitat, and life history all influenced flowering time, and habitat and life history were also phylogenetically conserved across 208 spp. Congeners differed in habitat more often than noncongener pairs, and also overlapped more in flowering time. Habitat shifts were not associated with shifts in flowering time in congeners, despite mean peak flowering time differences across habitats, and phylogenetic conservatism in habitat use. Congeners that differed in both habitat use and life history, however, did have the greatest difference in peak flowering dates. Habitat shifts likely play a role in local coexistence of close relatives, but our data do not support habitat-mediated changes in phenology as a possible mechanism. Experimental approaches may elucidate the role of phenology, resource competition, pollinators, and other associates in mediating coexistence of congeners at our coastal California field site.

Key Words: close relatives, congener, ecological speciation, eflora records, flowering phenology, heterospecific pollen transfer, phylogenetic, sympatry.

Understanding the drivers of plant flowering time provides insights into the ecology and evolution of plants (Sargent and Ackerly 2008) and their conservation (Sherry et al. 2007; Davis et al. 2010). Flowering phenology is a highly variable trait that determines the timing of resource use, the shared use of, or competition for, pollinators, as well as reflects plant responses to changing environments (e.g., Morales and Traveset 2008; Davis et al. 2010). In 2009, Levin proposed that habitat shifts should pave the way for ecological speciation. New habitats might cause phenological divergence in conspecifics through plastic responses to different conditions, and diverged phenologies across habitats – with divergent selection across habitats – could reduce gene flow across habitats and thus promote specia-

tion (Winterer and Weis 2004; Antonovics 2006; Savolainen et al. 2006).

The same effects of habitat shifts might cause flowering time divergence in heterospecifics that could influence speciation or local coexistence. Levin (2006) suggested that habitat shifts could be associated with increased rates of lineage diversification. If heterospecifics, especially close relatives, share cues that induce flowering, but cues come at different times seasonally across habitats, then habitat shifts may reduce co-flowering in heterospecifics without changes in flowering cue traits (Davies et al. 2013). For example, plants often start flowering in response to drying conditions (Rathcke and Lacey 1985; Warren et al. 2011; Ivey and Carr 2012; Li et al. 2016); thus, plants in drier habitats could be expected to flower earlier than close relatives in wetter

habitats. Moreover, annuals often occupy drier habitats than perennials and can differ in flowering time (e.g., Hall and Willis 2006), thus we also consider life history effects on flowering time in addition to habitat effects.

Co-flowering species may interact if they facilitate or compete for pollinator services (e.g., Moeller 2004; Agrawal and Fishbein 2008; Morales and Traveset 2008; Tur et al. 2016), if they hybridize, share floral predators, suffer reduced seed set from heterospecific pollen receipt or loss of pollen to heterospecifics (Arceo-Gomez and Ashman 2014, 2016; Toll and Willis 2018; Christie and Strauss 2020) or if they have peak resource use at similar times (Jensen et al. 2019). Moreover, co-flowering in (congeneric) close relatives in particular has been shown to result in greater rates of heterospecific pollen transfer and reproductive interference (Arceo-Gomez and Ashman 2016; Christie and Strauss 2020). Many of these effects would select for mechanisms that reduce co-flowering.

Here, we ask: what are the relative contributions of habitat, life history, and phylogeny as predictors of flowering time overlap between species, and do habitat shifts increase phenological divergence, as suggested by Levin (2009)? To address the specific challenges of close relatives, we also examine: 1) whether habitat and life-history shifts in congeners result in divergence in flowering time, potentially facilitating local coexistence; and 2) whether habitat shifts are more likely to occur in congeneric close relatives.

Habitat shifts may potentially be a mechanism that could result in changes in flowering time (Mallet et al. 2014). Variation in temperature, photoperiod, and moisture are all important cues for flowering time (Rathcke and Lacey 1985; Eckhart et al. 2004; Marques et al. 2004), and may be phylogenetically conserved (Davies et al. 2013). Co-flowering may be triggered by shared environmental cues like moisture availability or temperature (e.g., Diekmann 1996; Pau et al. 2011), which can differ across habitats (Franks et al. 2007; Jentsch et al. 2009; Levin 2009; Ivey and Carr 2012; Jordan et al. 2015; Anacker and Strauss 2016). Alternatively, co-flowering may be hard-wired, if species share flowering cues like daylength (Marques et al. 2004; Li et al. 2016), which is relatively insensitive to environmental conditions. Thus, for species using photoperiod as the predominant flowering cue, habitat shifts would not be associated with phenological divergence.

Phylogenetic conservatism in cues for flowering may also determine flowering time and flowering time overlap, especially in close relatives (Davies et al. 2013; Li et al. 2016; Lessard-Therrien et al. 2014; Anacker and Strauss 2014). Several studies find phylogenetic signal in plant phenology among large groups of plant species (Davies et al. 2013; Li et al. 2016; Lessard-Therrien et al. 2014), meaning that constraints to phenological plasticity and adaptations may limit phenological divergence among

relatives (Willis et al. 2008; Davis et al. 2010). Moreover, beyond phylogenetic signal in flowering time across diverse species, sister and closely related species often have similar flowering periods (Mosseler and Papadopol 1989; Ferguson and Jansen 2002; Debussche et al. 2004; Anacker and Strauss 2014; Li et al. 2016).

Using field-collected phenology data and flora databases of the Jepson Manual 2 and Jepson eFlora (Jepson Flora Project 2021), we explore the association of habitat and phylogeny with flowering phenology overlap at our field site, with a specific focus on congeners.

MATERIALS AND METHODS

Field Site

The Bodega Marine Reserve (BMR) is located in Sonoma County (38.3070°N, 123.0660°W) and is part of the University of California Natural Reserve System. BMR covers 362 acres along and adjacent to the California coast north of San Francisco. Because two continental plates meet along the San Andreas Fault through the reserve, BMR is also rich in discrete habitat types, despite being a small reserve. There are sand dunes, coastal grasslands/prairie, wetlands, and rocky coastal bluffs that receive salt spray. Some of these habitats differ in parent material and in moisture availability (Anacker and Strauss 2016).

Flowering Time

We used two flowering time datasets: first, the phenology of 59 native forb species in the field (hereafter, 'field data') and second, we collected flowering time data for 208 forb species that occur at the field site from the Jepson Manual 2, also available on the Jepson Flora Project (2021). The latter flowering periods are presented by month and are based on a combination of herbarium records and knowledge of the author of the species description (Baldwin, Jepson Herbarium, personal communication). These 208 species consisted of the 59 observed in the field plus 149 more present at the reserve; hereafter, 'Jepson data'. Using the 59 species observed in the field and for which we also had Jepson data, we asked how well our field observations were correlated with reported phenology from the Jepson data.

Field-based flowering time was censused from January to September 2011, covering the Mediterranean climate flowering season. Observations were collected in biweekly surveys of 59 native species of flowering plants across the reserve, including the most abundant flowering plants that year. We recorded the Julian date of flowering onset and then the number of flowers, fruits, and buds on up to 30 individuals of each species at each census period. We determined the date of peak flowering by selecting

the date when the most flowers in the population were open. Peak flowering can be less erratic between years than beginning or end of flowering (e.g., CaraDonna et al. 2014), and reflects the maximum number of flowers open for pollen donation and receipt. We used Julian dates for analyses, as all species at BMR start flowering after January 1 and finish flowering before the end of the long dry Mediterranean summer, well before December 31; thus, circular statistics (Morellato et al. 2010) for flowering dates were not required.

Our data reflect what species are doing at our field site, but 59 species is not a large sample size for the questions we wished to ask, especially with regard to congeners. At the time of this study in 2012, flora-based flowering time data by month were available for 208 of the 295 native forb species at BMR (71.5%) from the Jepson Manual 2; they thus reflect a much larger phylogenetic temporal and spatial scale than our data collections. To estimate peak flowering from the Jepson data, we used the midpoint of recorded flowering time. All flowering time data were converted to Julian dates.

One might expect large discrepancies between our temporally and spatially limited observations and the coarse range-wide data (see Discussion in de Keyser et al. 2017). However, we found that for the 59 species for which we had both field and Jepson phenology estimates, field peak flowering time was significantly and biologically meaningfully correlated with the Jepson data ($r = 0.68$, $P < 0.001$). This result gave us some confidence that estimates from the Jepson could be used to address our questions, and provided a larger sample size encompassing the majority of the native forb community at the BMR (208 of 259 species). Moreover, our results (below) are always in accordance across the field and Jepson data sets, though the field data suffer from a lack of power in some cases.

Habitat Use and Life History

The BMR contains discrete habitat types – coastal grassland and sand dunes – that flank each side of the San Andreas fault that runs through the reserve. In addition, there are seeps, and a freshwater marsh, as well as rocky outcrops and coastal bluffs close to the ocean exposed to extensive salt spray. In a previous study, we found that some of these habitats differ in soil moisture levels (e.g., rocky outcrops are much drier than marsh or bluff habitats; Anacker and Strauss 2016), an important flowering cue. Each of the 208 native forb species was assigned to one of the habitat types based on their occurrence at BMR by botanical experts and reserve managers at that time – Peter Connors and Jackie Sones. Species that occupied more than one habitat were assigned to the habitat in which they were most common; because these habitats are quite discrete and different, all species studied could be assigned primarily to one habitat. At this relatively small reserve (146 ha),

habitats were within the foraging distances of many abundant pollinators, notably those recorded for *Bombus spp.* (e.g., Jha and Kremen 2013).

Plant life history (annual/perennial), which is also known to affect flowering time (e.g., Fitter and Fitter 2002) was extracted from the Jepson Manual 2. Biennial species were coded as perennial.

Phylogenetic Tree

We built a phylogeny for the 208 native forb species based on molecular sequences for three genes (ITS, *matK*, and *rbcL*), downloaded from GenBank (Benson et al. 2012). We supplemented missing sequence data with sequences taken from congeners. The gene by species matrix is available from the Dryad digital repository (<https://doi.org/10.5061/dryad.qfttdz0hqand>). Sequences were then aligned using MUSCLE.

For the phylogeny, we first used the software program Phylomatic (Webb and Donoghue 2005) to generate a partially resolved topology that was used subsequently as a topological constraint tree. This phylomatic tree was based on a recent Angiosperm Phylogeny Group tree (R20100428). We then conducted a maximum likelihood analysis in RAxML (Stamatakis 2006), using the phylomatic tree as a topological constraint, a GTRCAT model, and 100 bootstrap replicates. The resulting RAxML tree was fully dichotomous with branch lengths in substitutions per site. Due to the size of the gene matrix, we used the RAxML tree to fix the topology during divergence time estimation in BEAST (Drummond et al. 2012). We constrained several nodes using fossil calibrations from Bell et al. (2010; listed below) with an arbitrary standard deviation of 0.1 Ma. We ran a single MCMC chain for 10 million generations, sampling every 1000 generations. We repeated the analyses twice and combined the resulting posteriors to assure convergence of the posterior distribution. From the combined BEAST posterior, a maximum clade credibility tree was made and uploaded to the open access repository Figshare (<https://doi.org/10.6084/m9.figshare.15135450.v1>).

Fossil Calibrations

Seedplant, 325; Apiaceae, 33; Asteraceae, 44; Boraginaceae, 59; Brassicaceae, 24; Convolvulaceae, 20; Crassulaceae, 41; Cucurbitaceae, 20; Fabaceae, 56; Gentianales, 71; Iridaceae, 32; Liliaceae, 48; Montiaceae, 83; Nyctaginaceae, 13; Onagraceae, 20; Papaveraceae, 112; Plumbaginaceae, 27; Polemoniaceae, 35; Ranunculaceae, 65; Rubiaceae, 56.

Statistical Analyses

To test for phylogenetic signal in peak flowering time, we calculated Pagel's λ , which provides a more robust measurement than other metrics (Münchmueller 2013), using “fitcontinuous()” function in the R package *geiger* (Harmon et al. 2008).

We also estimated phylogenetic signal in habitat use and life history, using Pagel’s λ for discrete traits using “fitDiscrete()” function in the same package; lambda values near zero indicate no phylogenetic signal, whereas values near one indicate strong phylogenetic signal. All analyses above and below were repeated for the field data and the Jepson data sets.

We next tested if phenology was correlated with habitat use and life history, while accounting for phylogenetic non-independence using the `phylANOVA()` function from the *phytools* package (Revell 2012). To disentangle the contributions of phylogeny, habitat, and life history on peak flowering time, we also used variance partitioning (Desdevises et al. 2003; Peres-Neto et al. 2006; Gonçalves-Souza et al. 2014). We fit an ecological trait model (habitat + life history), a phylogeny model, and a trait + phylogeny model. To represent the phylogeny in linear terms, we decomposed the phylogeny into a set of principal coordinates (PCs) using the “PVRdecomp” function of the *PVR* package. We regressed each PC against peak flowering time, retaining those that were significantly related at $\alpha = 0.05$. For the 59-tip phylogeny, we retained just one PC; for the 208-tip phylogeny, we retained 10 PCs. Next, we derived the four constituent components via subtraction of the R^2 values from the three regression models, as described by Desdevises et al. (2003).

To address the patterns specifically in close relatives, we conducted a set of analyses on congener pairs; the mean estimated divergence of congeners for the field and Jepson data was 6.9 and 11.7 My, respectively. First, each congener pair was placed into one of four “shift” categories: none (shared habitat and life history), habitat only, life history only, and both (differ in habitat and life history); the counts were compared using a Chi-squared test. We then compared the peak flowering time difference, calculated as $\text{abs}[\text{peak flowering species A} - \text{peak flowering species B}]$, with the type of shift for every congener pair using a one-way ANOVA. Because of the pairwise nature of the data, we reduced our degrees of freedom in the statistical test to equal the number of unique genera, rather than the number of pairs. For the field data, the number of congener pairs was 17, and the number of unique genera was 10; for the Jepson data, the number of congener pairs was 116, and the number of genera was 42. All analyses were performed in R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Effects of Phylogeny, Habitat, and Life History on Phenology

Peak flowering time, habitat affinity, and life history all contained moderate to strong phylogenetic signal (Fig. 1, Table 1). Peak flowering time was also

TABLE 1. PHYLOGENETIC PATTERNS IN FLOWERING TIME, HABITAT-USE, AND LIFE HISTORY (ANNUAL OR PERENNIAL) USING PAGEL’S λ . *P < 0.05; **P < 0.01; ***P < 0.001.

	Field data	Jepson data
Number of species	59	208
Flowering time λ	0.60***	0.41***
Habitat-use λ	0.68*	0.83**
Life history λ	0.96*	0.81***

related to both habitat and life history for both datasets, even after accounting for phylogenetic non-independence (Fig. 2).

Phylogeny also explained substantial variation in peak flowering time using variance partitioning methods (Table 2). For the Jepson data, the total variation in peak flowering time was partitioned into a traits-only component (17%), a phylogeny-only component (17%), a shared trait + phylogeny component (16%), and unexplained variation (49%). The 17% shared trait + phylogeny component reflects the correlation of phylogeny and traits (Fig. 2). The 17% trait-only component suggests that the relationship between the traits and peak flowering times remained after accounting for phylogenetic autocorrelation, consistent with the results of the `phylANOVA` tests. The 17% phylogeny-only component represents effects of shared evolutionary history. In total, we explained up to 51% of the total variation in peak flowering time using habitat, life history and phylogeny as predictors, and all of these terms were included in the best model to explain peak flowering time.

Flowering Similarity in Congeners

On average, median divergence in peak flowering time was 20 d less between congeners than non-congeners, based on all pairs of flowering species [congeners: 33 mean, 28 median d divergence; non-congeners: mean 53 d, median 49 d divergence; $t = 3.3$, 16.8 df; $P < 0.01$ from the field data; similarly, 31 mean and 30 d median for congeners, vs 48 mean and 45 d median from Jepson data; $t = 7.1$, 117.6 df; $P < 0.001$). Importantly, 15% of congeners had complete overlap in peak flowering time, while only 9.8% of noncongeners did, based on the larger Jepson dataset.

Despite more similar peak flowering times overall, congeners still exhibited about a 3 wk divergence in peak flowering. The majority of congener pairs differed in one or both habitat or life history traits (70.5% for field data; 51.7% for Jepson data; Table 3).

The number of congener pairs in each shift category (habitat shift only, life history shift only, habitat and life history shift, no shift) differed from the null expectation of the relative frequency of habitats and life history for the Jepson data ($P < 0.001$), and showed the same trend in the field data (P

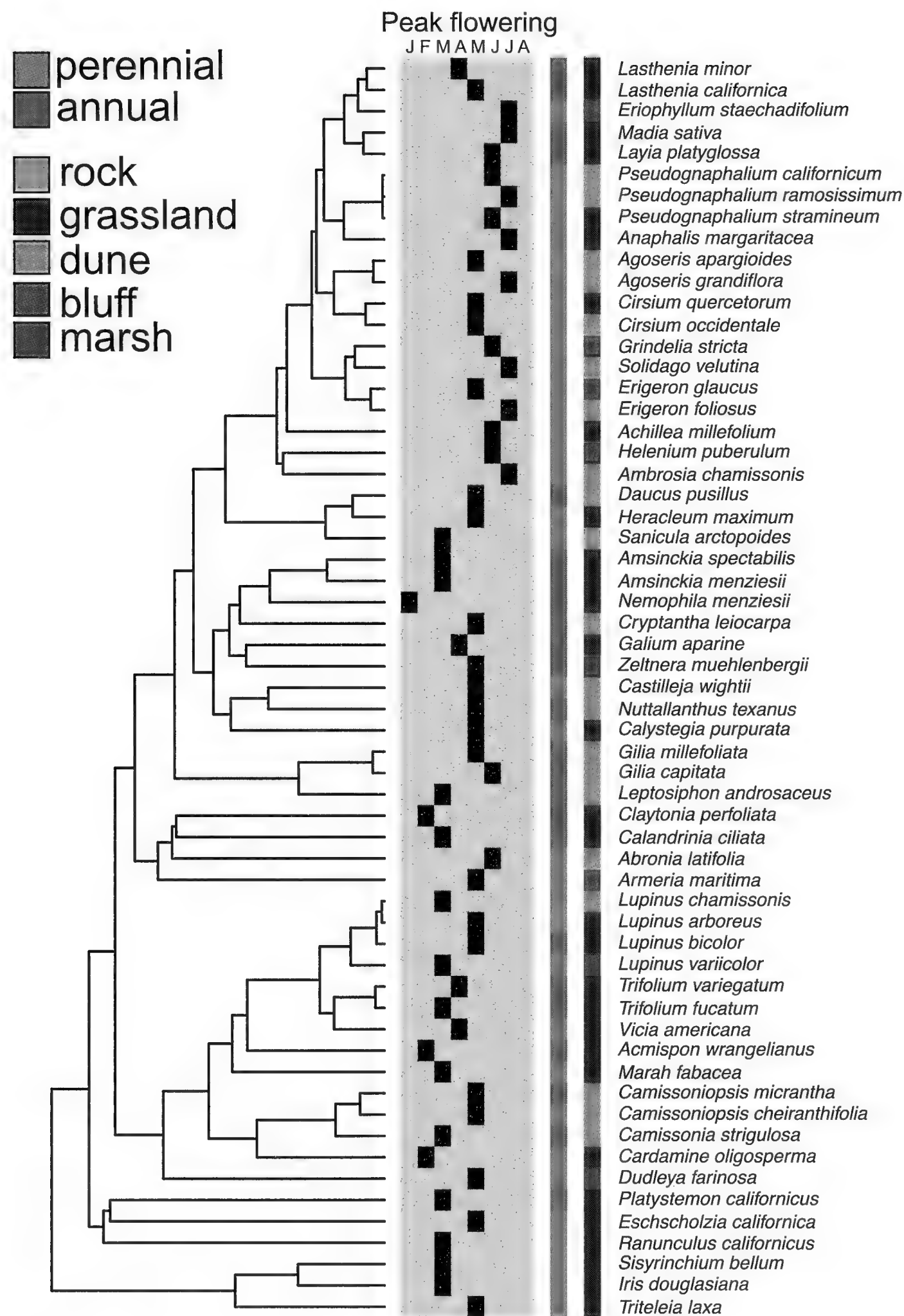


FIG. 1. Phylogeny of 59 plant species indicating field-collected flowering time, habitat affinity at BMR, and life history.

= 0.12; n = 17 pairs for the field data vs. n = 116 for the Jepson data). Congeners were more likely to partition habitats than noncongeners. Habitat shifts were ~2 times more common than life history shifts, but alone did not affect peak flowering (Table 3; 29.6 vs 29.3 d for field data and 23.6 vs 23.2 for Jepson data), counter to Levin’s (2009) hypothesis.

DISCUSSION

Plant species that flower at the same time overlap in use of resources and pollinators, and shared floral predators. If flowering time is phylogenetically constrained, either by shared cues or indirectly through shared habitats, then shared flowering time can 1) increase resource competition, 2) facilitate pollination or increase competition for pollinators, and 3) increase opportunities for heterospecific pollen transfer. Levin (2006, 2009) suggested that

habitat shifts might cause plants to diverge rapidly in flowering time, assortatively mate, and thereby become reproductively isolated through ecological speciation (e.g., Savolainen et al. 2006; Osborne et al. 2019); he provided a convincing review of divergence in phenology with habitat shifts within species (see also Stam 1983; Winterer and Weis 2004; Gavrillets and Vose 2005, 2007). We explored whether flowering time was phylogenetically conserved, and whether habitat shifts might provide a way for heterospecifics to partition flowering time, potentially reducing negative effects on each other’s fitness resulting from reproductive and competitive interactions.

We found evidence for phylogenetic conservatism in habitat use, as many other studies have found (e.g., Cavender-Bares et al. 2006; Kraft and Ackerly 2010), and that species occupying different habitats differed in mean flowering time, after taking phylog-

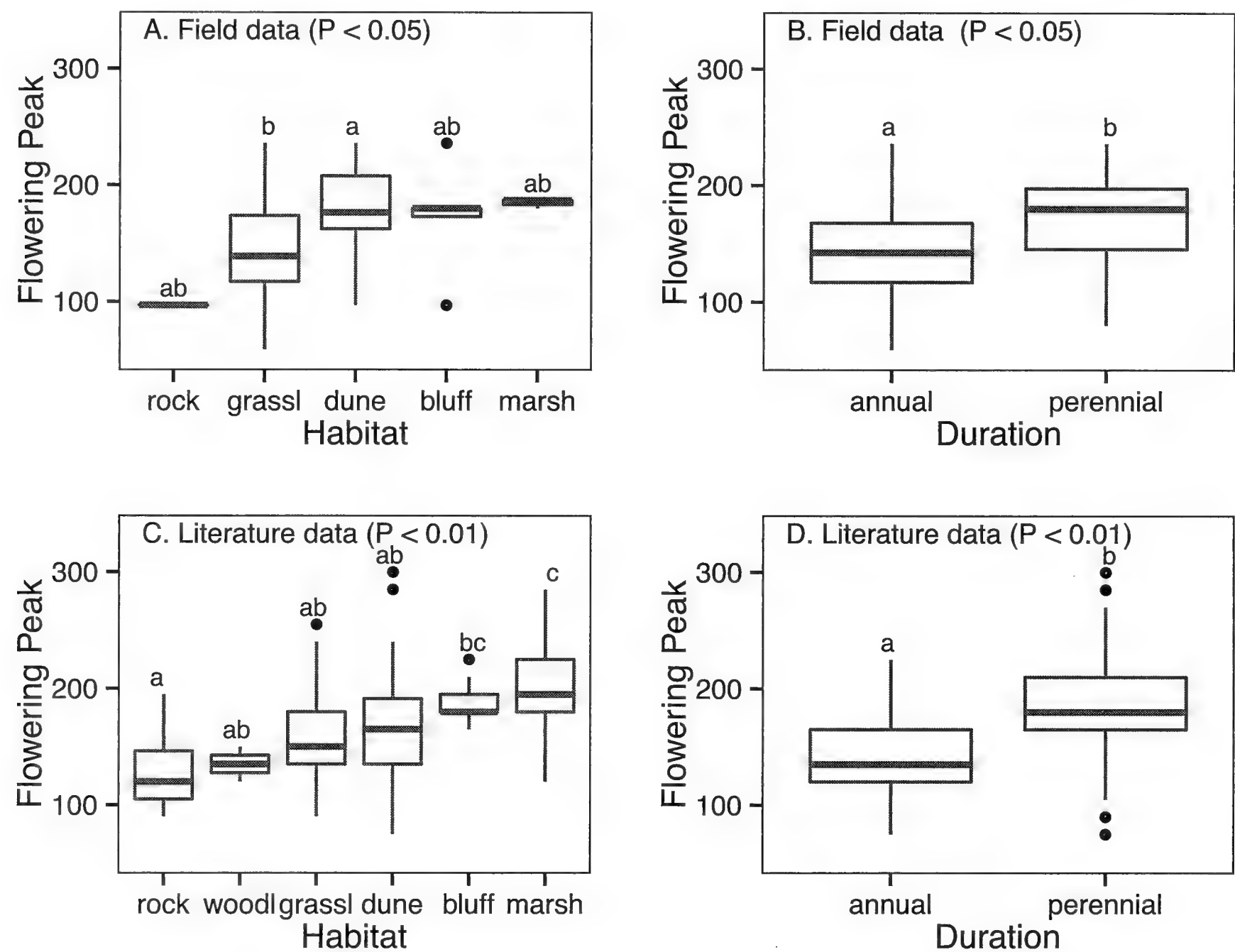


FIG. 2. Flowering time by habitat affinity and life history for native species at Bodega Marine Reserve. Letters indicate significant differences based on Tukey’s HSD.

eny into account. Thus, both phylogeny and habitat influence flowering time. The predictive power of phylogeny, both in terms of phylogenetic signal in habitat use and life history and on its own, was quite large. Peak flowering time had phylogenetic signal of a magnitude consistent with the findings of previous studies of plant phenology (Staggemeier et al. 2010; Davies et al. 2013; Seger et al. 2013; Lessard-Therrien et al. 2014; Li et al. 2016; Fig. 1; Table 1).

Despite the fact that we found divergence in mean peak flowering time across habitats, consistent with Levin’s hypothesis, an analysis focusing only on congeners at BMR found that congeners differing in habitat alone did not diverge more in peak flowering time than those sharing the same habitat type. Congeners were more likely to completely overlap in peak flowering time than were noncongener pairs,

and their peak flowering times were less diverged than those of non-congeners. Thus, it is likely that conservatism in flowering times outweighed habitat effects on phenology at the congener level.

Phylogenetic signal in flowering time may arise through several pathways: first, flowering cues may be conserved (Rathcke and Lacey 1985); if based on daylength, they may be largely invariant across a range of habitats (Marques et al. 2004; Li et al. 2016). Initiation of flowering in Mediterranean ecosystems may be especially cued on daylength, as other systems with less predictable rainfall show the importance of daylength in determining flowering time (e.g., Cortes-Flores et al. 2017). We did not find evidence for a second pathway to conserved flowering time in congeners: phylogenetic signal in the types of habitats where species grow, and habitat-specific

TABLE 2. PLANT FLOWERING TIME PARTITIONED AMONG HABITAT AND PHYLOGENETIC COMPONENTS. Adj. R² values for partition of variance (bottom of table) were obtained via subtraction of the Adj. R² values of the top model. Shared and unexplained components are untestable (Peres-Neto et al. 2006)

	Field data Adj. R ² (P)	Jepson data Adj. R ² (P)
Method without partition of variance		
Traits (Habitat + life history)	21.9 (<0.01)	33.6 (<0.001)
Phylogeny	34.1 (<0.001)	33.5 (<0.001)
Traits and phylogeny	49.0 (<0.001)	50.9 (<0.001)
Method with partition of variance		
Traits only	15.0 (<0.01)	17.4 (<0.001)
Shared (Traits + Phylogeny)	6.9 (–)	16.2 (–)
Phylogeny only	27.2 (<0.001)	17.3 (<0.001)
Unexplained	51.0 (–)	49.1 (–)

TABLE 3. DIFFERENCE IN PEAK FLOWERING DATE (FLOWERING TIME DISTANCE), HABITAT SHIFTS, AND LIFE HISTORY SHIFTS AMONG CONGENER PAIRS. The number of congener pairs in each shift category (habitat shift only, life history shift only, habitat and life history shift, no shift) differed from the null expectation based on the relative frequency of habitats and life histories; these patterns were stronger for the Jepson data ($P < 0.001$), but showed the same trend in the field data ($P = 0.12$).

Shift type	Field data		Jepson data	
	n	Mean flowering time distance (SE)	n	Mean flowering time distance (SE)
None	6	29.2 (7.10)	50	23.4 (2.65)
Habitat only	7	29.6 (9.20)	35	23.6 (3.33)
Life history only	2	17.5 (3.50)	9	38.3 (7.12)
Both	2	76.0 (0)	22	58.0 (6.42)
Total	17		116	

flowering times. Plants often initiate flowering in response to abiotic cues, such as drydown (Rathcke and Lacey 1985; Warren et al. 2011; Ivey and Carr 2012; Li et al 2016), and we had data showing that habitats differed in water content of soils at BMR (Anacker and Strauss 2016); thus, plants in drier habitats could be expected to flower earlier than close relatives in wetter habitats (Eckhart et al. 2004; Jentsch et al., 2009, Mazer et al. 2021, this issue). However, we did not find that close relatives diverged in peak flowering time when they occupied different habitats. A caveat is that our sampling intervals were coarse, and we would not have detected the 3-d divergence of two *Clarkia* congeners occupying slightly different habitats (Mazer et al 2021). In our study, divergence in flowering time was greatest in congener pairs differing in both habitat and life history.

Another caveat to our study is that we could not take into account the influence of time since divergence between congeners on their flowering time divergence because phylogenetic relationships/sequence data for congeners at Bodega Bay were not available for many species. When these relationships become known, an analysis taking into account the effects of time since divergence between congeners on the magnitude of phenological shifts would be very informative.

Phenological overlap and the role of phylogeny in constraining peak flowering time may impose challenges to or facilitate coexistence, especially for close relatives (Runquist and Stanton 2013; McEwen and Vamosi 2010; Weber and Strauss 2016). For congeners, challenges to coexistence lie in how resources are partitioned, how much pollen is exchanged, and how enemies are shared between ecologically similar species with similar habitat preferences. Our prior work has shown that congeners compete more intensely at the BMR field site than do less closely related taxa, and also that congeners exhibit spatial overdispersion at the field site (Anacker and Strauss

2014, 2016). Challenges to co-flowering could entail in increased competition for pollinators (Morales and Traveset 2008; Albrecht et al. 2016) or greater heterospecific pollen transfer that reduces fitness (Grossenbacher and Stanton 2014; Runquist and Stanton 2013; Arceo-Gomez and Ashman 2016; Christie and Strauss 2020). Thus, phenological overlap in close relatives may favor habitat shifts to reduce any of these types of negative interactions. Spatial sorting would be the mechanism through which habitat divergence mitigates the negative effects of flowering overlap. We found that congeners were more likely to partition habitats than non-congeners in this study. Only additional field experiments can further elucidate the underlying mechanisms driving habitat partitioning among congeners at BMR.

Our results are also important from a methodological perspective. We had good agreement of field-collected phenological data for 59 species from diverse families taken at a local field site –where character displacement in peak flowering time might be occurring at microallopatric scales– with phenological records for the same species from the Jepson Manual 2/ Jepson eflora summarized over the range of the species. This study reinforces the conclusions of a growing body of research demonstrating the value of survey resources like field guides and herbarium records in the study of plant phenology (e.g., Davis et al 2015; Willis et al. 2017; Love et al. 2019).

Another caveat in interpreting our results is that total flowering overlap may not coincide with estimated peak flowering overlap. That said, we chose peak flowering because it was more consistent from year to year than other aspects of phenology in previous long-term phenological studies (CaraDonna et al. 2014, and it represents when most of the flowers in the population are open.

CONCLUSIONS

Levin (2009) suggested that habitat shifts might drive ecological speciation through a reduction in gene flow across habitats. He did not find support for this hypothesis across species; habitat shifts were not associated with lineage diversification on islands or mainlands in a literature review (Levin 2006). We find that flowering time is influenced by phylogeny, habitat use and life-history across 208 native forb species occupying our field site. However, we did not find evidence that habitat shifts caused phenological divergence in close relatives, counter to the initial expectations. Habitat shifts were, however, more prevalent in congener pairs than noncongener pairs at BMR, despite overall phylogenetic signal in habitat use. These results suggest that microsympatric congeners interfere with each other. Because congeners share flowering times, flower color and morphology, heterospecific pollen deposition and reproductive interference could be an underappreci-

ated mechanism contributing to selection for habitat divergence in close relatives, in addition to traditionally considered forces of resource competition.

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PHENOLOGY OF ANNUAL DORMANCY RELEASE AND ITS ASSOCIATION WITH FRUIT SET OF *DIRCA OCCIDENTALIS* (THYMELAEACEAE)

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ABSTRACT

Weather and climate may influence the phenology of *Dirca occidentalis* A.Gray (Thymelaeaceae) in ways that impact reproductive success. *Dirca occidentalis* blooms during winter, when the likelihood of entomophily may be low. Based on preliminary observations that the timing of dormancy release and growth resumption varies over years and among shrubs within years, we quantified fruit set among flowers that formed at different times and examined whether annual variation in autumnal precipitation and temperature during autumn and winter are associated with phenology. Fruit set was determined during 2007–2008 through 2011–2012 by tracking 37,461 flowers near or at anthesis early, midway, and late within the blooming period of *D. occidentalis*. In addition, measures of phenology of 18 individual shrubs were made each December 29, January 26, and February 23 of the five blooming periods, and fruit set of these shrubs was determined. Fruit set was low (<5%) among flowers present early (December 26–January 2), but increased significantly in all blooming periods, to as high as nearly 30%, among flowers at anthesis later. Phenology ratings, and lengths of newly formed stems and leaves, on December 29 increased linearly as the amount of precipitation from October 1–December 15 of the same year increased. Phenology ratings on February 23 increased linearly with increasing air temperature from November 1–February 23. Rankings of phenology of the 18 shrubs were highly correlated over years, and fruit set of individual shrubs over years was 1% to 52% and increased linearly as growth resumption and flowering became later. Our results demonstrate that low autumnal precipitation is associated with delayed growth resumption and flowering, which corresponds with increased fruit set of this rare species.

Key Words: climate, *Dirca occidentalis*, endemism, fruit set, Mediterranean, phenology.

Plant phenology is closely associated with weather and subject to changes in climate (Parmesan and Yohe 2003; Menzel et al. 2006a, b; Cleland et al. 2007; Willis et al. 2017; Yost et al. 2020). As sessile organisms, plants are robust indicators of their environments. Phenophases of plants are strongly affected by interannual variability in weather and climatic patterns, and changes in environmental factors influence vigor, competitiveness, and survival (Walther et al. 2002; Davis et al. 2015). Because temperature and water relations are among the most important determinants of plant phenology (Menzel et al. 2006a, b; Gill et al. 2015), year-to-year thermal and hydric variation can alter the timing of phenological events (Menzel et al. 2006a, b; Cleland et al. 2007; Inouye 2008).

Regions with Mediterranean climates, such as coastal California, are characterized by warm, dry summers and cool, wet winters. Some plants in Mediterranean climates undergo drought deciduousness in summer. Leaves desiccate and may abscise as plants become quiescent during extended dry periods before rainfall in autumn or winter promotes the resumption of shoot growth (Griffin 1973; McCreary 1990). Annual variations in weather can shift the onset of growth resumption, and the timeframes of growth (McCreary 1990; Prieto et al. 2008) and flowering (Wolkovich et al. 2013). Earlier flowering in winter and spring has been associated with elevated cool-season temperatures and autumnal

precipitation (Prieto et al. 2008). While phenological asynchrony between pollinators and their food plants caused by climate change is not the norm (Hegland et al. 2009; Forrest 2014), temporal mismatches between some pollinators and early-flowering plants have been documented (Mommott et al. 2007; Tylianakis et al. 2008; Renner and Zohner 2018). Such misalignments can reduce pollination and fecundity. Species distributed narrowly in small populations may be particularly prone to extirpation amid climate change (Aitken et al. 2008).

Dirca occidentalis A.Gray, a member of the only extant genus of the plant family Thymelaeaceae indigenous to the continental United States, is endemic to the California counties of San Mateo, Santa Clara, Contra Costa, Alameda, Marin, and Sonoma (Graves and Schrader 2008). Known as leatherwoods, *Dirca* spp. are shrubs with arborescent forms, precocious yellow flowers in late autumn through early spring, flexible stems, and fibrous bark with high tensile strength (Hudson 2019).

Among the species of *Dirca*, only *D. occidentalis* occurs in a Mediterranean climate. Plants typically are clustered in the understory of sloped woodlands, but chaparral (Ackerly 2004) and semi-riparian habitats also may include the species. Reproduction is primarily sexual (Graves and Schrader 2008). Copious hermaphroditic flowers are borne annually, but the single-seeded drupes of *D. occidentalis* are rarely observed (Johnson 1994). Because shrubs

bloom during winter, low temperatures may limit the activity of insect pollinators, a phenomenon observed in other members of the genus (Williams 2004; Graves 2008). The phenology of *D. occidentalis* has received little attention, however, and our preliminary observations indicate considerable variation among individuals. As a narrowly endemic species in a family that is not prevalent in the flora of North America, *D. occidentalis* merits research to improve our understanding of its phenology and how climate change may affect the species.

Dirca occidentalis is found in numerous habitats within Jasper Ridge Biological Preserve (JRBP) in San Mateo County. The species is most abundant in evergreen woodlands, but also is found in riparian areas along creeks, scrub, and chaparral. At 61.6 to 211.5 m above sea level, the 483-hectare preserve covers the northern half of Jasper Ridge within the San Francisquito Creek watershed. Data from local weather stations show average annual precipitation from 1975 to 2004 was 652 mm. About 90% of annual precipitation occurs from November through April; the warmest months are August and September, when mean temperature is about 17.5°C; and the coolest months are December and January, when mean temperature is about 10.5°C.

Because of summer deciduousness, the functional lifespan of leaves of *D. occidentalis* in chaparral communities at JRBP is four months (Ackerly 2004). *Dirca occidentalis* we observed in woodlands, scrub, and chaparral at JRBP became quiescent during summer. Further, the timing of phenological events marking growth resumption appeared asynchronous among individuals, and fruit set appeared to vary markedly among plants and over years. Based on these preliminary observations, our objectives were to examine whether annual variation in autumnal precipitation and temperature was associated with phenology, and to quantify fruit set among flowers that formed at different times within the long blooming period of the species. Our approach was to quantify fruit set by tracking tens of thousands of flowers that formed early, midway, and late in the annual blooming period for the species over five consecutive winters. In addition, the phenological statuses of the same 18 plants were recorded on the same three dates during those five winters, and the fruit set of those plants was determined. We provide evidence that the timing of growth resumption among individual shrubs following summer quiescence is highly variable, that precipitation and temperature are strongly associated with phenology of *D. occidentalis*, and that differences in phenology are closely associated with variation in fruit set.

METHODS

Fruit Set

Fruit set of *D. occidentalis* was documented during five consecutive winters, 2007–2008 through 2011–

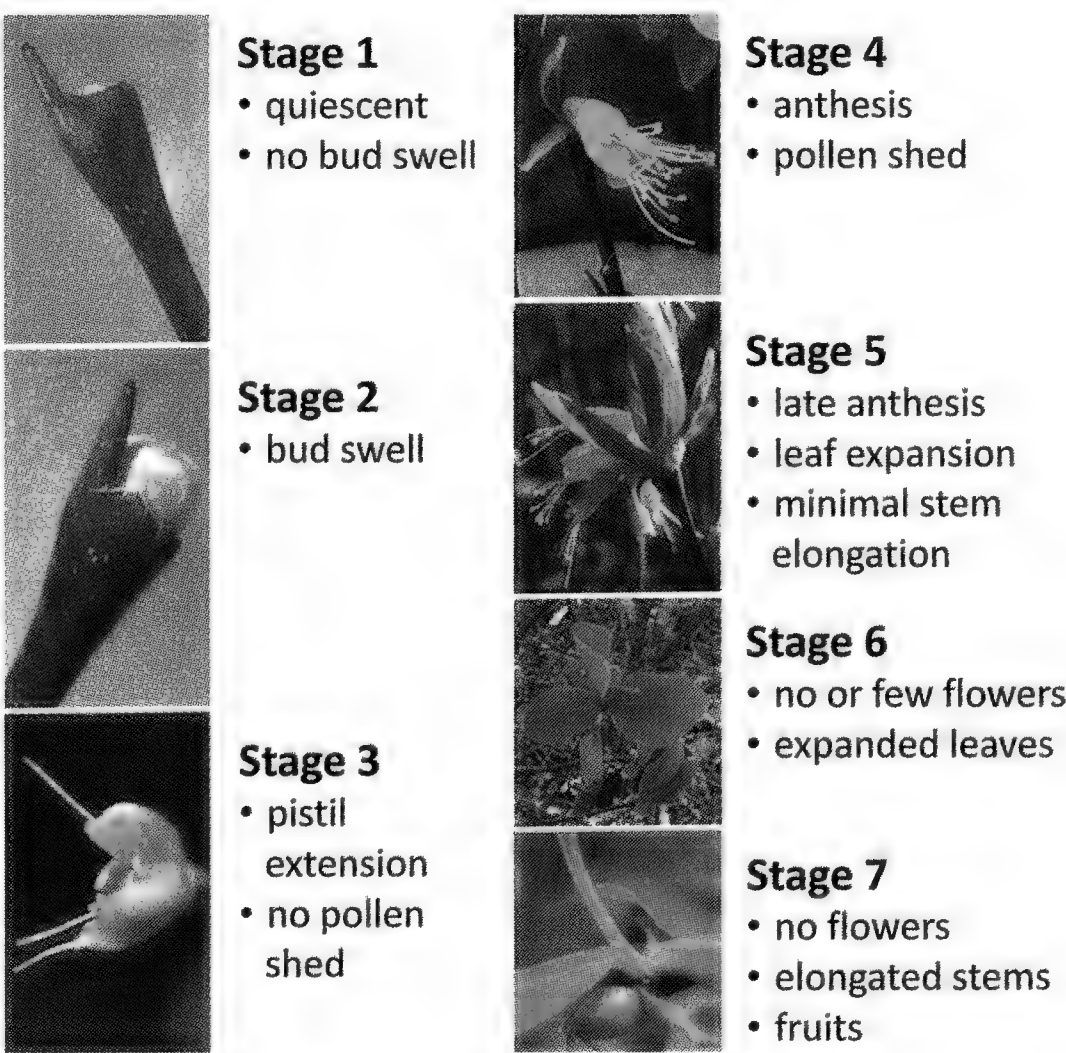


FIG. 1. The phenology of *Dirca occidentalis* was rated based on a seven-level scale. Stages 1, 4, and 7 represented quiescence, anthesis, and post-floral shoot and fruit development, respectively. Photographs by W.R. Graves.

2012. Numerous locations throughout JRBP were scouted each winter to include *D. occidentalis* in the range of habitats in which the species occurs. Most *D. occidentalis* were found in the understory of wooded, north- and east-facing slopes, but individuals also were adjacent to waterways at the bottoms of slopes, and were scattered within upland chaparral communities. Dataloggers (iButton model DS1921G, Maxim Integrated, San Jose, CA) were used to record air temperature at 120-minute intervals 1.5 m above grade at 10 locations chosen to represent the habitats of the *D. occidentalis* included in this study. Although *D. occidentalis* in all habitats were included each winter, the individual plants on which fruit set was documented varied among winters and were not tracked. Flowers on shrubs of various sizes were tagged. Because shrubs of average size usually support copious flowers, only small fractions of the total flowers per shrub typically were tagged.

Within each winter, inflorescences were tagged to allow subsequent determination of fruit set. Only inflorescences with flowers that had developed at least to the stage of pistil extension (Stage 3, Fig. 1) were tagged, and no inflorescences with flowers past anthesis were tagged. Most tagged inflorescences comprised flowers at anthesis (Stage 4, Fig. 1), with extended pistils and stamens, and anthers that had begun to shed pollen. Tagging was done within one-week periods early (December 26–January 2), mid-way (January 23–30), and late (February 20–27) during the flowering period for the species to determine whether fruit set differed during the three periods when flowers were tagged. Air-temperature data during each of these weekly periods and the week immediately following them were obtained

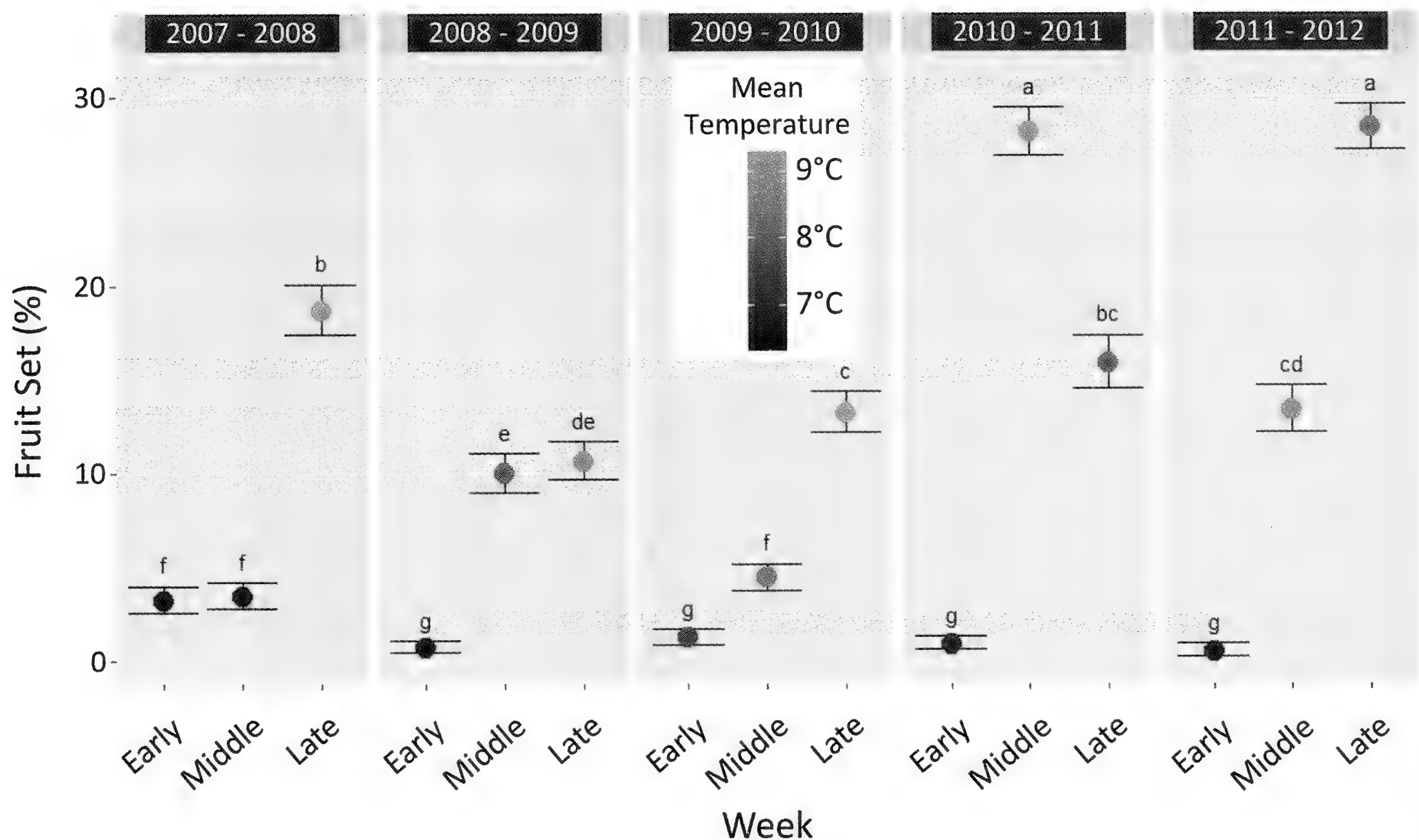


FIG. 2. Fruit set, the percentage of flowers that formed fruits, of *Dirca occidentalis* was determined during five consecutive blooming periods, 2007–2008 through 2011–2012. Flowers present during early (December 26–January 2), middle (January 23–30), and late (February 20–27) portions of the flowering periods were tagged and checked after approximately one month for fruit development. Each symbol represents mean fruit set among the number of flowers shown in Table 1 for the corresponding week. The shades of blue of the symbols indicate the mean air temperature during the week of tagging and the week that immediately followed. Symbols are bracketed with 95% confidence intervals, and letters represent all pairwise comparisons within and among five blooming periods.

from the dataloggers to encompass the timeframes of anthesis. Averaged over the five blooming periods, mean temperatures during these three two-week periods were 6.6°C, 8.1°C, and 8.7 °C, respectively, with maximums of 14.0°C, 16.8°C, and 18.3°C. Among the five blooming periods, only during 2010–2011 were temperatures higher in the middle week (mean = 9.3°C, maximum = 22.6°C) than during the late week (mean = 8.0°C, maximum = 19.1°C).

Tags were pieces of adhesive-tape 2–3 cm long. A tag was attached to the stem immediately below the node of each chosen inflorescence. Care was taken to avoid contact with flowers during tagging, and to position tags such that they could not touch flowers. Tags were removed when data were recorded after approximately one month. Because flowers that do not set fruits abscise one to two weeks after anthesis, fruits were readily noted as swelling ovaries. Data on fruit set, determined as the percentage of flowers that led to a developing fruit, were analyzed to determine effects of the five blooming periods and the individual weeks of tagging flowers.

Data were fit by using a generalized linear model (GLM) in RStudio (R version 3.5.2; Rstudio, PBC, Boston, MA). Estimated dispersion with the binomial family of the GLM function was 0.726, so the quasibinomial family was used (dispersion parameter

of 0.891). Estimated marginal means were calculated with the *emmeans* package, and predicted probabilities along with their 95% confidence intervals were plotted (Fig. 2). A compact letter display of all pairwise comparisons was calculated using the *cld* function of the *multcomp* package. Results were plotted with symbols that indicate air temperature during flowering by using the *ggplot2* package.

Phenology

Thirty plants of *D. occidentalis* that represented the diverse habitats in which the species occurs at JRBP were chosen to track phenology during the winters of 2007–2008 through 2011–2012. Twelve of these plants were disregarded during the study because they died or were damaged by felled trees or herbivory significantly enough that obtaining data over five winters was not possible.

The 18 remaining plants were rated each winter to document their developmental statuses on December 29, January 26, and February 23. A seven-level scale was used, where 1 denoted a quiescent plant, 4 denoted full anthesis, and 7 denoted that flowering had ended, fruits were developing, and that newly formed stems had elongated substantially (Fig. 1). In addition, the lengths of newly expanding leaf blades and elongating stems were measured to the nearest

TABLE 1. INFLORESCENCES OF *DIRCA OCCIDENTALIS* TAGGED (AND THE CORRESPONDING NUMBER OF FLOWERS) MONITORED, TO DOCUMENT FRUIT SET. Tagging was done during three one-week periods in five consecutive flowering periods. The three weeks were during early (December 26–January 2), middle (January 23–30), and late (February 20–27) portions of the flowering period of the species.

Week	Blooming period	Inflorescences	Flowers
Early	2007–2008	642	1948
	2008–2009	836	2585
	2009–2010	749	2224
	2010–2011	865	2712
	2011–2012	500	1519
Middle	2007–2008	747	2315
	2008–2009	861	2641
	2009–2010	919	2885
	2010–2011	972	2983
	2011–2012	725	2198
Late	2007–2008	770	2330
	2008–2009	909	2776
	2009–2010	998	3016
	2010–2011	635	1910
	2011–2012	1096	3419

0.1 mm by using a Verner caliper. After thoroughly examining the developing tissues, what appeared to be the five longest leaves and stems of each plant were measured.

Fruit set of these plants was also estimated. Rather than tagging inflorescences, approximately one month after the floral period of a plant ended, 30 floral nodes along stems were checked for the presence of developing fruits. The 30 floral nodes were chosen in the same way for each plant. First, the tallest branch of the shrub was identified. Then, the uppermost floral node along that branch was checked, followed by the 29 floral nodes that immediately subtended it. Whether a node was floral was determined easily because each flower present within an inflorescence resulted either in a fruit or in a distinctive scar on the stem where the pedicel had abscised. The number of flowers that the 30 inflorescences had comprised was determined as the number of fruits plus the number of scars, and fruit set was calculated as the percentage of flowers that led to a fruit. The 18 plants were ranked annually based on mean ratings (Fig. 1) on the three dates from 1 (earliest to develop, leading to the highest mean phenology rating) to 18 (latest to develop, leading to the lowest mean phenology rating). Means of the five annual mean rankings for each plant were used in a linear regression analysis to examine whether mean ranking was associated with mean fruit set over the five blooming seasons.

Environmental data from 2007 to 2012 were obtained from the 10 dataloggers and a weather station at JRBP. Total precipitation from October 1 to December 15 was used in regression analyses to determine whether precipitation was associated with the mean phenological rating, and mean leaf and

stem lengths, of the 18 plants on December 29. The relationship of air temperature and phenology was examined with a linear regression analysis of mean temperature from November 1 to February 23 and mean phenology rating of the 18 plants on February 23.

Means of the three phenology ratings recorded each year for the 18 plants was determined to examine their relationship to fruit set. Because residual plots showed heteroskedastic variance of error, fruit-set data were square root-transformed, which corrected the non-constant variance. The function lmer() from the R package lme4 was used to fit a mixed linear model to this set of repeated measures collected over the five annual blooming periods. Random intercepts for plants were assumed independent, and Restricted Maximum Likelihood was the estimation method. In addition, Spearman rank correlations determined by using the function cor() from base R were used to assess consistency in how the 18 plants ranked from year to year based on their mean phenology ratings.

RESULTS

Fruit Set

Ample flowers were available to tag during each of the three weeks of all five blooming periods. Total inflorescences tagged per weekly period ranged from 500 to 1096, which corresponded to 1519 to 3419 flowers (Table 1). The total number of inflorescences tagged per annual blooming period ranged from 2,159 to 2,666. The overall total number of flowers included in this study across the three weekly periods and five annual blooming periods was 37,461.

During most blooming periods, fruit set was higher among flowers blooming during the middle week (January 23–30) and late week (February 20–27) than in the early week (December 26–January 2) (Fig. 2). The only exception to this was in 2007–2008, when there was no difference between the early and middle week. Across all five blooming periods, mean fruit set was <5% for flowers present during the relatively cool early week, whereas mean fruit set was highest among flowers blooming in the late week during three of the five periods (Fig. 2). In 2008–2009, however, there was no difference in mean fruit set between the middle and late weeks, and, in 2010–2011, fruit set was highest in the middle week. The highest mean fruit sets, nearly 30%, were in the middle week of 2010–2011 and in the late week 2011–2012 (Fig. 2).

Phenology

Among the 18 plants that could be monitored during all five blooming periods, mean phenology rating on December 29 increased linearly with seasonal precipitation from October 1 through December 15 (Fig. 3). For each 1-cm increase in

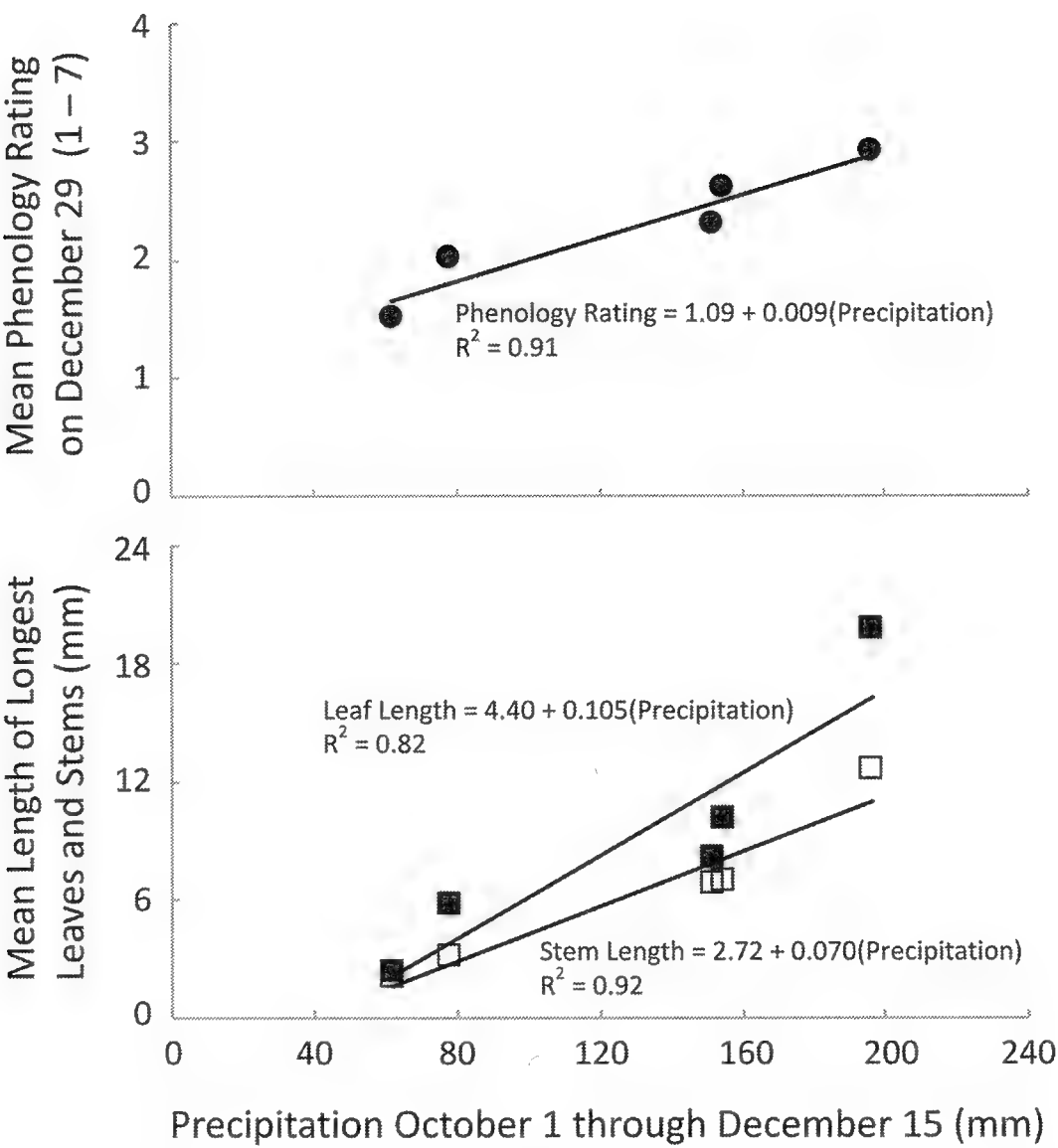


FIG. 3. Mean phenology rating (upper plot) and mean length of the longest newly formed leaves (filled squares) and stems (open squares) of the same 18 plants of *Dirca occidentalis* on December 29 are shown with total precipitation from October 1 through December 15 in five consecutive years. Each symbol represents one year, 2007–2011. The phenology rating scale depicted in Fig. 1 was used.

precipitation, mean phenology rating increased by 0.009 ($R^2 = 0.91$, $P < 0.05$). Leaf expansion and stem elongation also increased linearly with total precipitation from October 1 through December 15 (Fig. 3). Mean length of the longest leaves increased by 0.105 mm per 1-cm increase in precipitation ($R^2 = 0.82$, $P < 0.05$), and mean length of the longest stems

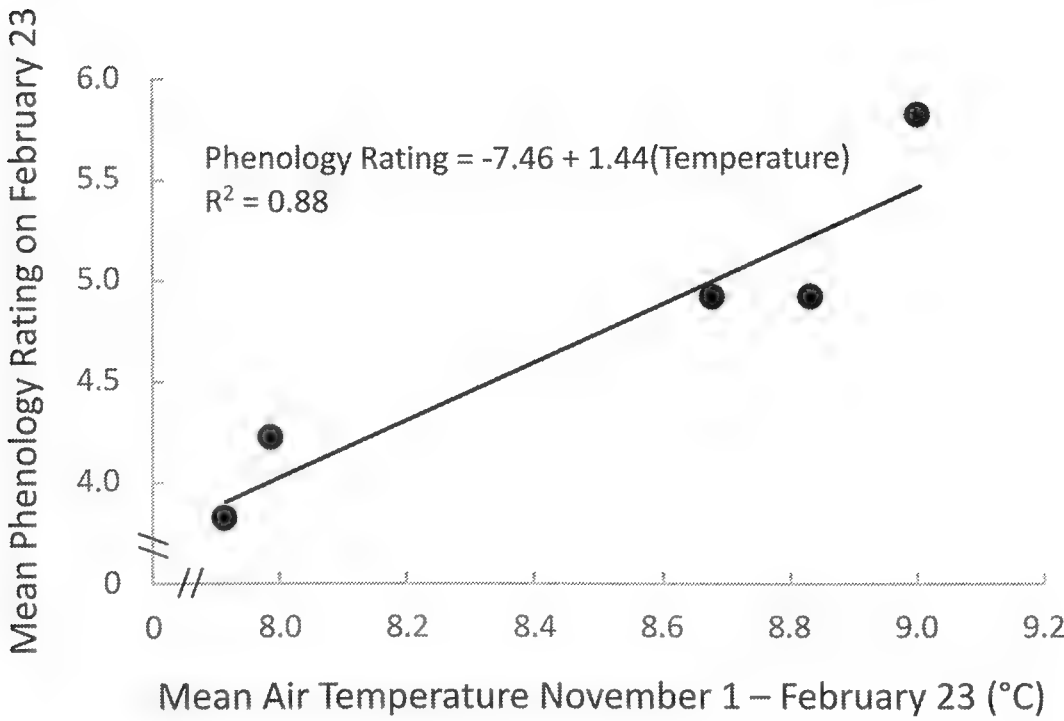


FIG. 4. Mean phenology rating on February 23, based on the scale depicted in Fig. 1, of the same 18 plants of *Dirca occidentalis*. Each symbol represents the overall mean phenology rating of five yearly means (2008–2012) for the 18 plants. The linear regression of overall mean phenology rating and mean air temperature from November 1 through February 23 of each blooming period (2007–2008 through 2011–2012) is depicted.

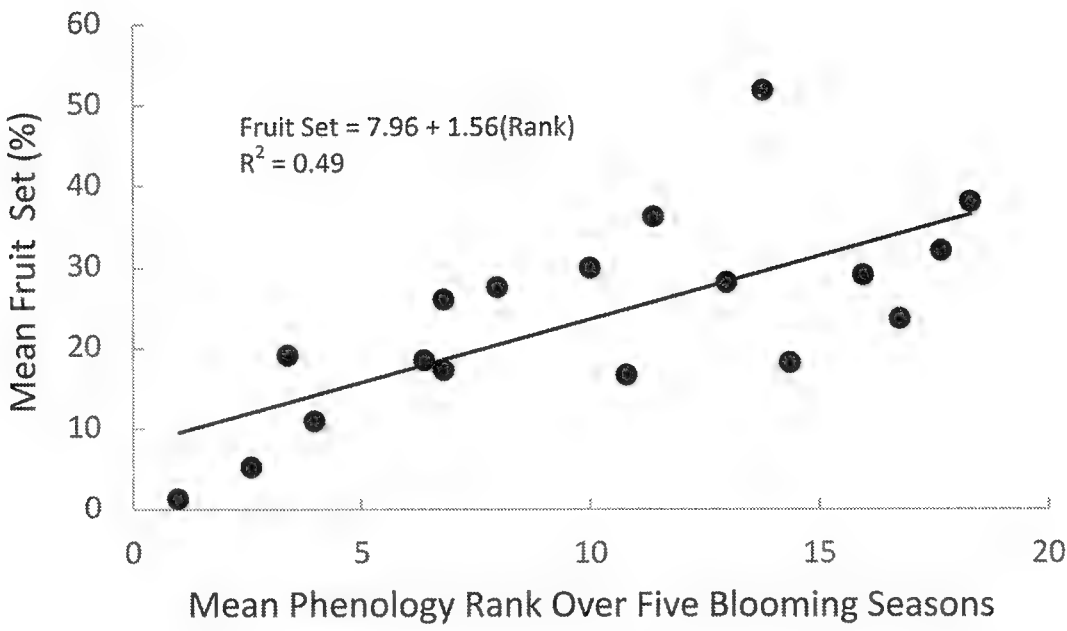


FIG. 5. Mean fruit set of the same 18 plants of *Dirca occidentalis* is shown with the corresponding mean phenology rating for each plant over five blooming periods. The phenology rating scale depicted in Fig. 1 was used on December 29, January 26, and February 23. Plants were ranked annually based on mean ratings on the three dates from 1 (earliest to develop, leading to the highest mean phenology rating) to 18 (latest to develop, leading to the lowest mean phenology rating), and means of the five annual mean rankings for each plant were determined.

increased by 0.070 mm per 1-cm increase of precipitation ($R^2 = 0.92$, $P < 0.01$). Mean phenology rating on February 23 increased linearly ($R^2 = 0.88$, $P < 0.05$) by 1.44 per 1°C increase in temperature (Fig. 4).

Differences in phenology of these 18 plants were associated with their fruit set. Mean fruit set over blooming periods increased linearly ($R^2 = 0.49$, $P < 0.01$) with later flowering and vegetative development as indicated by increased mean phenology rank (Fig. 5). The mixed linear model showed an effect of mean phenology rating ($P < 0.001$) and the fixed effect of year ($P < 0.001$). The variance of the plant random effect was 0.6213 with a residual error of 1.5694. The estimate for the effect of phenology rating was -0.8817 ($t = -6.482$, $df = 35.8$, as determined by Satterthwaite approximation). The adjusted intra-class correlation coefficient (ICC) was 0.284.

Spearman rank correlations of yearly rankings of the 18 plants based on their mean phenology ratings were strong and positive among all 10 pairwise combinations of years. Correlations ranged from 0.77 to 0.94, and the median of the correlations was 0.90.

DISCUSSION

This research provides new information regarding the autecology of *D. occidentalis*. First, our data provide new insights regarding variation in the phenology of this species. Individual shrubs varied in phenology within blooming periods, and phenology of the population of shrubs at JRBP collectively shifted across years. The fact that flowering precedes the resumption of vegetative growth in late autumn or winter in *D. occidentalis* accounts for the second insight from this work: the variation in the timing of flowering is associated with differences in fruit set.

Third, there is evidence that rainfall and air temperature are associated with phenology and fruit set.

The numerous shrubs of *D. occidentalis* within the 483-hectare JRBP bloom asynchronously over approximately four months. Individual shrubs did not bloom throughout that period. Rather, flowering was highly synchronous within individual shrubs that were floral during different portions of time from December through February. The phenological statuses during winter of the 18 shrubs we monitored over five blooming periods were highly variable. For example, their statuses on January 26 ranged from Stage 1 to Stage 7 (Fig. 1) in 2009, 2010, and 2012, and the ranges were Stage 1 to Stage 6 in 2008 and Stage 2 to Stage 6 in 2011. Individual shrubs were floral (Stages 3–5 or 6) for approximately two to three weeks, and individual flowers were at anthesis for up to one week. The type of asynchronous flowering we observed was described as plastic by Rodríguez-Pérez and Traveset (2016). Most shrubs of *D. occidentalis* provided flowers in particular abundance in late January and late February (Table 1), but we were able to tag at least 1519 flowers each year from December 26–January 2, and we observed flowers several weeks before late December in most years, as well as flowers after late February in all years.

Although clusters of plants within some habitats tended to bloom rather synchronously, we frequently observed one to a few outlier plants within larger clusters that did not flower at the same time as the majority. Based on such observations, and the fact that *D. occidentalis* can spread clonally (Graves 2004), future studies could examine genetic relatedness of individuals within clusters and whether the degree of floral synchrony among closely positioned individuals influences fruit set. As the image depicting Stage 3 of our phenology rating scale illustrates (Fig. 1), *D. occidentalis* may be protogynous. Results of pilot studies we have conducted on pollination suggest the species is self-compatible, but that cross pollination bolsters fruit set and seedling vigor. Further research is needed to document the pollination biology of *D. occidentalis* and the consequences of the mode of pollination on fruit seed, seed viability, and seedling vigor.

The timing of annual resumption of growth and flowering among individuals of *D. occidentalis* varies for unresolved reasons that may include genetic variation (Graves and Schrader 2008) and microsite variation in air temperature and edaphic characteristics that influence soil water content. Pratt and Mooney (2013) observed intraspecific variation in phenophase timing of *Artemisia californica* Less. provided different quantities of water in a garden plot. Such plastic asynchrony of flowering may provide a degree of resistance to effects of climate change on reproductive success. The strongly positive Spearman rank correlations provide evidence for consistency across years in the degree of earliness of

individuals. We noted that the same individual shrub ranked as the earliest to pass through the phenological stages we defined (Fig. 1) each blooming period, and only two different shrubs were ranked second earliest across the five years. In addition, the same shrub ranked latest of the 18 in four of the five years. Greater variation in rankings was evident for individual shrubs that were neither extremely early nor late to develop. Evaluation of the range of factors that may influence annual variation in phenology among individuals of *D. occidentalis* merits additional research.

Based on our preliminary observations that *D. occidentalis* at JRBP is floral over long periods comprising late autumn and all of winter, and a report that fruits rarely form (Johnson 1994), we questioned whether time of anthesis is related to fruit set. We conclude that it is. Among large samples of flowers tracked by tagging them while at anthesis, fruit set was at least tenfold higher for flowers tagged during the late week than for flowers tagged during the early week, and the greatest percentage of fruit set was from flowers tagged during the middle or late week within each of the five annual blooming periods (Fig. 2). Consistent with these findings, we observed that fruit set increased linearly among the 18 individuals we monitored as phenology of annual growth resumption became later (Fig. 5). The negative effect of phenology rating when fruit-set data were analyzed with a mixed linear model further demonstrates increased fruit set with later flowering and resumption of vegetative growth. The adjusted ICC from the linear mixed effect model indicates fruit set of individuals is modestly positively correlated across years, i.e., a low to moderate tendency for individuals to be consistent in their fecundity.

As a species that is putatively entomophilous, the increase in fruit set with later flowering could be linked to increased pollinator activity later in the blooming period (Corbet et al. 1993). Air temperature, which often is strongly associated with the time of flowering (Abu-Asab et al. 2001), tended to be lowest during weeks when flowers led to few fruits (Fig. 2). Pollinator activity could explain this observation. Low and mean temperatures we recorded were below the threshold that Williams (2004) associated with pollinator visits to *D. palustris*, which flowers in late winter and early spring in eastern North America. In contrast, relatively high fruit set was coincident with high temperatures during the middle week of 2010–2011 and during the late week of all other blooming periods (Fig. 2). We therefore posit that individuals of *D. occidentalis* that resume annual growth and bloom relatively late are more likely to set fruits than shrubs that serve as harbingers by resuming growth and blooming relatively early, i.e., before mid to late January.

How precipitation can influence pollinator activity also merits consideration. Precipitation events when flowers are at anthesis are most likely during relatively wet blooming periods, which tended to

hasten flowering of *D. occidentalis* (Fig. 3). By damaging flowers and disrupting pollinators, rainfall, along with low temperature, might suppress fruit set. Water from rainfall can alter the osmotic potential of pollen grains, causing them to burst (Corbet and Plumridge 1985; Lawson and Rands 2019), and pollen may not adhere to and germinate on wet stigmatic surfaces (Ortega et al. 2007). Rainfall also disrupts pollinator activity by removing floral volatiles, physically limiting pollinator movement, and altering nectar rewards through dilution (Totland 1994; Cnaani et al. 2006; Lawson and Rands 2019). Furthermore, the activity of small pollinators that putatively service *Dirca* is suppressed during periods of low solar radiation typical of precipitation events (Willmer 1983; Kilkenny and Galloway 2008). The interplay of environmental factors, particularly precipitation, air temperature, and solar radiation, on insects that pollinate *D. occidentalis* merits elucidation.

In addition to variation among individual plants in the phenology of annual growth resumption and flowering, this research also provides evidence that the temporal window of dormancy release, shoot development, and flowering of *D. occidentalis* shifts from year to year at JRBP. The extent of annual shoot development evident on December 29, first manifested by swelling buds (Fig. 1), was positively correlated with the amount of precipitation from October 1 to December 15. Regression analyses predict increasing phenology ratings, greater extension of newly formed stems, and longer newly developing leaves with increasing autumnal precipitation (Fig. 3). The influence of precipitation on floral phenology varies among species (Ogaya and Peñuelas 2004; Peñuelas et al. 2004; Crimmins et al. 2010) and may differ regionally (Abu-Asab et al. 2001). In a study of two Mediterranean shrub species that bloom in autumn, Prieto et al. (2008) found that an imposed drought treatment in late spring and summer delayed flowering of *Globularia alypum* L., while there was no effect on *Erica multiflora* L. Precipitation was more predictive than temperature of the timing of annual vegetative development of three of four species of woody plants in California analyzed by Mazer et al. (2015). Their results demonstrate the importance of precipitation timing. For example, the release of pollen from *Baccharis pilularis* DC. was hastened and delayed by precipitation during December and February, respectively, while, in *Quercus lobata* Née, bud break was either promoted or delayed by precipitation, depending on the month during winter when it occurred. In a study of *A. californica*, which is more broadly distributed than *D. occidentalis*, Pratt and Mooney (2013) found that changes in water availability advanced and delayed flowering depending on the provenance along a 700-km gradient from which plants had been cloned. Based on these studies, additional research should build on the relationship we found between precipitation from October 1 to December 15 and the

phenology of *D. occidentalis* (Fig. 3). Additional research should further define how the amount and timing of precipitation affect dormancy release and flowering, and the roles of temperature and soil edaphic conditions that influence shrub water status. Based on our results, we hypothesize that the annual resumption of shoot growth of *D. occidentalis* is triggered relatively early during blooming periods with high precipitation in autumn (Fig. 3), and that the progression through stages of seasonal development (Fig. 1) accelerates as temperatures in November through February increase (Fig. 4). A robust test of this idea would comprise shrubs from genetically distinct populations on the peninsula south of San Francisco, the East Bay, and the North Bay (Graves and Schrader 2008).

This research provides the first data documenting the phenology of the rare, endemic shrub, *D. occidentalis*. Our findings demonstrate marked phenological variation among individual shrubs, differences in fruit set based on floral phenology, and the association between environmental factors and phenology. Although this research provides valuable new information, additional research on the fate of initiated fruits is needed. The fraction of these fruits that mature and contribute viable seeds that germinate and give rise to new individuals remains unknown, as is how climate change will affect *D. occidentalis*. If autumns and winters in the Bay Area of California become wetter and warmer, our results suggest that flowering of *D. occidentalis* would be hastened, potentially resulting in lower fruit set and recruitment of new individuals unless there are simultaneously shifts in the activity of effective pollinators. In contrast, while drier autumns and winters might delay the flowering period and increase fruit set, reduced water availability in winter and early spring could suppress seed germination and seedling establishment, as well as the vigor of mature shrubs.

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PARTITIONING GENETIC AND ENVIRONMENTAL COMPONENTS OF PHENOLOGICAL VARIATION IN *QUERCUS DOUGLASII* (FAGACEAE)

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ABSTRACT

Oaks (genus *Quercus*) often display a large range of phenotypic variation across many of their traits. The contribution of genetic and environmental sources, and their interaction, to this variation can be partitioned experimentally using common garden plantings in which several genotypes are grown in a single location and the phenotype of interest is measured. Due to their slow growth and complex genetic structure, oaks have rarely been grown to maturity in experimental conditions that would allow partitioning phenotypic variation in this traditional way. Here we present results from trees growing in two established experimental gardens of *Quercus douglasii* Hook. & Arn. planted in 1991. Data from these common gardens are combined with additional data collected at the original provenance populations that served as acorn source locations. We surveyed phenological progression through the spring at both garden and field sites and found significant associations between fall and spring minimum temperatures and spring phenology, represented here as the date of bud break. A genetic component of phenological variation associated with the provenance sites was identified at both common gardens and accounts for 16.4% of the total variation observed among trees, while 68.2% of the variation can be attributed to environmental plasticity, plus a genetic \times environmental interaction that accounts for about 1% of bud break variation. We discuss the implications of these components of phenological variation in Blue Oak, especially with respect to climate change, local adaptation, restoration, and assisted gene flow.

Key Words: Blue Oak, common garden, environment, genetics, phenology, phenotypic variation.

California oaks show large variation in the timing of bud break and flowering across their range in any given year. This variation is commonly associated with both geographic differences among sites and local topographic variation within sites. Attempts to explain phenological variation in temperate trees tend to focus on analyses of weather variables such as temperature or precipitation, cumulative metrics such as growing-degrees, or geographic proxies such as latitude or elevation (Polgar and Primack 2011; Roberts et al. 2015). Among California oaks, previous research has shown that winter and spring temperature and precipitation are important drivers of spring phenology (Koenig et al. 2015; Gerst et al. 2017). These results are further confirmed by additional research reported in this special issue by Armstrong-Herniman and Greenwood (2021) and Koenig et al. (2021). Conflicting results among these studies concerning the effects of particular predictors suggest that phenological responses in these oaks may result from complex interactions, potentially integrated over more than one growing year. Additionally, the combination of multiple highly correlated weather variables, or especially weather and geographic variables, in a single phenological model makes identifying their independent effects even more difficult.

Temperature and the availability of water or sunlight prior to or early in the growing season are direct environmental drivers of phenology. Genetic phenological adaptation of populations to longer-term local conditions has often been found in previous research as well (Savolainen et al. 2007; Wilkinson et al. 2017; Dixit et al. 2020), including for the California oak *Quercus lobata* Née (Valley Oak) as reported by Wright et al. (2021) in this special issue. Genetic differentiation among populations is expected in a variable environment when environmental, population, or community interactions that depend on phenology have fitness effects. The timing of spring phenology (bud break and flowering) has several potential fitness effects for trees, via pollen limitation (Knapp et al. 2001), freezing damage (Augspurger 2013), and herbivory (Pearse et al. 2015). Direct influences of resource availability in the environment on phenological timing are a plastic response that allows individuals to respond to variation from year to year, including responses to progressive directional changes associated with climate change. Genetic adaptation to historical climate, on the other hand, limits the potential range of responses to the immediate environment. These limits can be adaptive if they dampen responses to short-term anomalies, but they also constrain the potential

to respond to directional change and can result in non-adaptive climate change responses and evolutionary lags (Browne et al. 2019).

Climate change simultaneously challenges California oaks with increased mortality from extreme events at the trailing edge of their distribution (Brown et al. 2018) and a potential dispersal lag at the leading edge that would prevent them from expanding into newly suitable locations (Serra-Diaz et al. 2014). The ability for oaks to respond to both of these challenges crucially depends on acorn production, which has been shown to be influenced by spring phenological timing, both as a result of flowering synchrony (Koenig et al. 2015) and herbivore pressure early in the season (Pearse et al. 2015). High fecundity in local populations at the trailing edge could preserve the adaptive potential of those populations against dieback due to extreme weather events by increasing genotypic diversity. Simultaneously, at the leading edge, greater acorn production can increase the rate of longer distance dispersal.

In the contexts of conservation and ecological restoration, climate change has given new impulse to debates over how to preserve the evolutionary potential of local populations while also encouraging migration into suitable habitats. Climate-informed assisted gene flow recommends sourcing seeds for restoration from sites that have historical climates more similar to the restoration site's predicted future climate, on the assumption that local adaptation to the historical location will make them better adapted in the new location compared to locally sourced seed (Aitken and Whitlock 2013). More recently, genome-informed assisted gene flow has also been suggested, which involves selecting seed sources based on empirically confirmed relationships between source genotypes and fitness measures, such as growth rate or reproductive success, at a target site (Browne et al. 2019). The restoration community has responded with skepticism to the suggestions for assisted gene flow due to a long-established preference for local seed sources in order to avoid disrupting existing patterns of local adaptation (McKay et al. 2005; Vander Mijnsbrugge et al. 2010). A lack of information concerning how much local adaptation there is among oaks, what the natural rates of gene flow are, and whether assisted gene flow is likely to disrupt locally adapted genotypes has left restoration specialists uncertain how to best proceed with restoration of degraded oak ecosystems in light of climate change (Spotswood et al. 2017; Baumgarten et al. 2020).

Reciprocal transplant and common garden experiments, in which individuals from several different local populations are grown and observed in one or more common environment, provide the clearest ways to address questions about the relative importance of local environment and genetic adaptation on phenotype (Clausen et al. 1941; Howe et al. 2003). Here we present results from established common

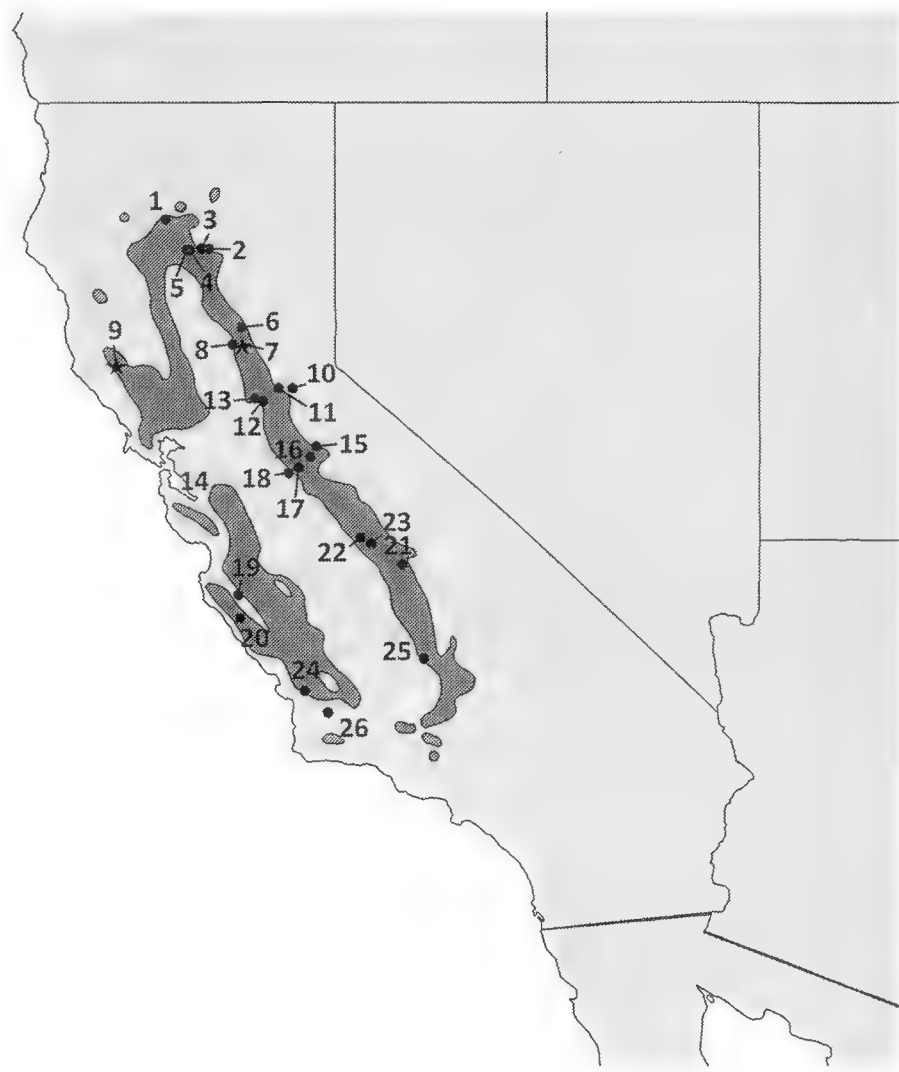


FIG. 1. The distribution of *Quercus douglasii* in California. The 26 acorn provenance locations and field survey sites used in this study are shown with black markers. Two common gardens are planted close to provenance 9 (Hopland) and provenance 7 (Sierra Foothills), indicated with stars on the map.

garden plantings of the California endemic blue oak (*Quercus douglasii* Hook. & Arn.), along with observations of adult trees at the original acorn provenance field sites sampled for the common gardens. Using spring bud break phenology as a readily observed measure of phenotypic differentiation, we examine the relative roles of local environment and genetics on the timing of bud break. We also investigate the roles of local weather at the garden sites and climate history at the provenance locations to identify how patterns of temperature and precipitation variation affect bud break phenology in blue oaks over both long and short timescales.

METHODS

Acorns for the common garden plantings were originally collected in 1990 at 26 provenance sites spanning the range of *Quercus douglasii* (Blue Oak), including the species' entire latitudinal range, as well as five transects across its elevational range in the Sierra Nevada (Fig. 1, Appendix S1). Acorns were collected from 10 maternal trees at each provenance site and grown together for the first year at a greenhouse location in Magalia, CA. In 1992, the one-year-old seedlings were then planted out at two common gardens, one in Mendocino County at the Hopland UC Research & Extension Center (N39.0137, W123.0959) and the other in Yuba County at the Sierra Foothills UC Research & Extension Center (N39.2467, W121.3173). The plantings followed a randomized complete block design consisting of ten blocks, each with nine sub-replicate seedlings

from each of the 26 provenance sites. Plots were weeded, but not irrigated during the first 2 yr of seedling growth and were protected by deer fences. In 2001–2002, six of the ten original blocks at each garden were thinned from nine to three sub-replicates per provenance. For additional details on the establishment of the garden plantings, refer to McBride et al. (1997). Trees were more than 25 yr old at the time of this study and only trees growing in the thinned blocks were surveyed, as growth in the blocks that had not been thinned was clearly reduced due to crowding. Mean tree height across all populations in the thinned blocks at Hopland was 3.79 m (SD = 1.15 m) and at Sierra Foothills was 3.67 m (SD = 0.93 m). Of the 468 trees originally planted in the six survey blocks at each garden, 13 had died by 2017 at Hopland and 105 had died at Sierra Foothills. Despite their age and size, flower production was only sporadically seen on some trees over several years of observation.

In addition to the common gardens, 21 of the original 26 acorn provenance sites were re-identified and surveyed for this study in 2017. In 2018, three additional provenance sites were identified for a total of 24. The exact original maternal trees could not be located using the collection records, but the sites selected to represent the provenance populations are within 4 km of the original collection trees and ± 50 m elevation. At each provenance field site, 7 to 12 trees at least the same size as the trees currently growing in the common gardens were selected for phenological observation.

Spring phenology was surveyed on each tree at the common garden and provenance sites in 2017 and 2018 using a bud break index that ranged from 0 to 8 (see Appendix S2 for descriptions of each index stage). Common garden sites were surveyed every 7 to 14 d between mid-February and late-April. Provenance field sites were surveyed a minimum of two times during the same period.

The phenological index was designed to show a linear progression during leaf out across index values 1–7. This was confirmed using common garden trees surveyed at high frequency. As a result, a linear model could be fitted to the periodic survey data and used to interpolate a common phenological stage for all trees even though they were not all surveyed at the same stage. Phenological index value 3 (bud break) is used as the common stage for all analyses reported here.

Using the phenological variation observed in the common garden plantings, we ran two-way ANOVA (R 3.5.2, R Core Team, Vienna, Austria) to partition the phenotypic variation in the date of bud break into separate environmental and genetic components. Common garden location \times observation year (four levels in total) represents the environmental component while acorn provenance location (26 levels) represents the genetic component. The interaction between them is a genotype-by-environment ($G \times E$) effect and was included in the model.

Next, combining phenological data from both common gardens and the 24 provenance field sites, we sought to explain the environmental and genetic components of variation in terms of weather and climate variations using linear regression models. For the environmental component, weather variables at the individual growing location (common gardens or field sites) were used from the current water year, which runs from October of the previous year to September of the growth year in order to capture precipitation patterns in California's Mediterranean-type climate. To identify the genetic component explained by adaptation to local climate, we used 30-yr climate means from 1951–1980 (the 20th century climate baseline) at the provenance collection sites.

Gridded weather and climate data were extracted from the Basin Characterization Model (BCM) for California, which models weather and hydrology using the PRISM weather models topographically downscaled to 270 m resolution (Flint et al. 2013). Monthly estimates of minimum and maximum temperature and precipitation (36 total raster layers) were used for both the study period 2017–2018 as well as the climate baseline 1951–1980 (Appendix S3). To maximize explanatory power (adjusted R^2) while reducing multicollinearity among the 72 weather and climate variables, we used automated AIC-based stepwise model selection (function *stepAIC* in the R package *MASS*). This was followed up by manually removing individual remaining variables to reduce multicollinearity (as measured by the variance inflation factor, VIF) and highlight important variables.

RESULTS

More phenological variation was found across the provenance field sites than was found within either of the gardens. Mean date of bud break for populations at the provenance field sites in 2017 spanned 32 d from March 6 to April 7 in 2017 and 78 d in 2018 from February 18 to May 7. At the Sierra Foothills common garden, the mean date of bud break among the provenance populations spanned 19 d from February 26 to March 17 in 2017 and 35 d from February 10 to March 17 in 2018. At the Hopland common garden, mean date of bud break spanned 23 d from March 12 to April 4 in 2017 and 25 d from March 15 to April 9 in 2018. The longer period covered by bud break in 2018 compared to 2017, especially in the Sierra Foothills common garden and provenance sites located in the Sierra Nevada foothills, may be associated with a cold snap with freezing temperatures in early March of 2018. When data from the two common gardens are combined, the variation in bud break timing in the gardens covered approximately the same range of dates as the field provenance sites (Fig. 2).

In addition to the difference in bud break timing between the Hopland and Sierra Foothills common gardens and some differences between the 2017 and

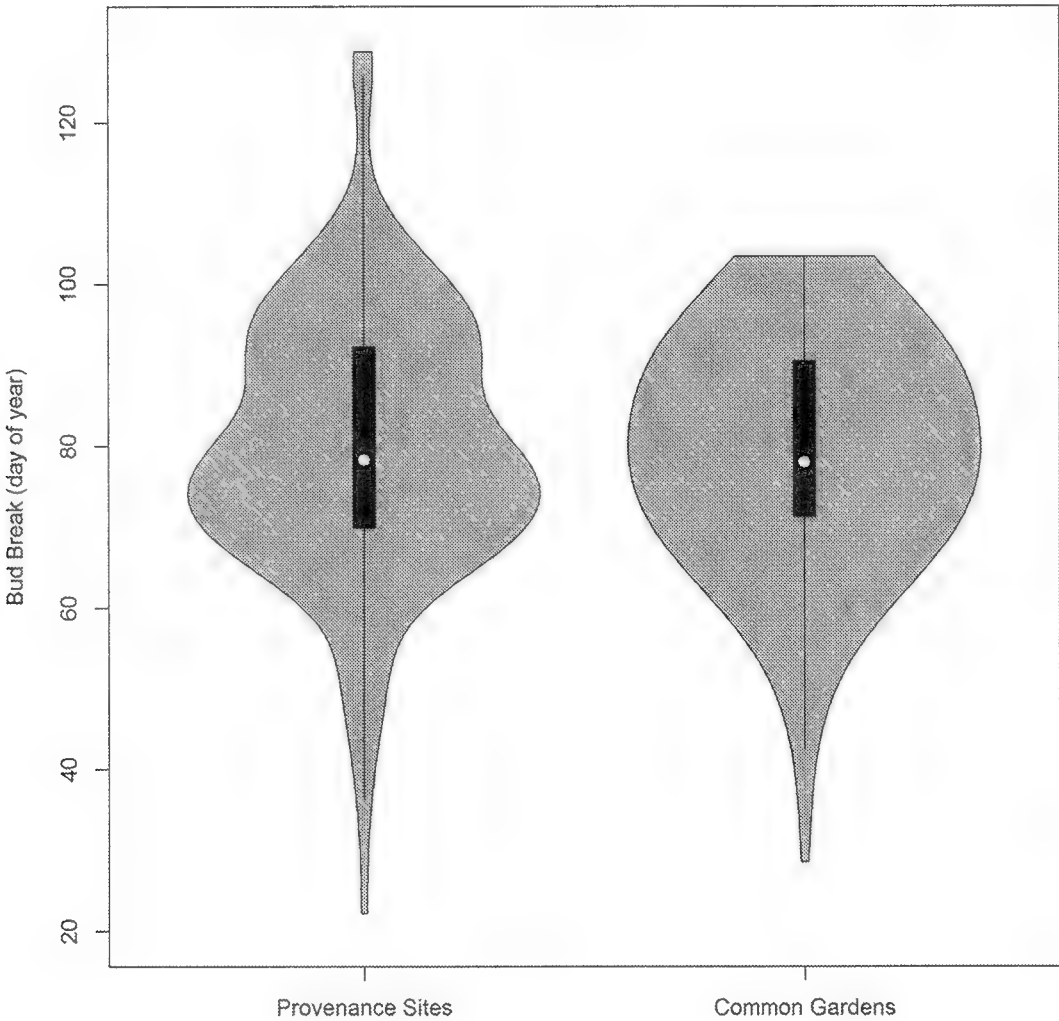


FIG. 2. Variation in the date of bud break in *Quercus douglasii* observed in 2017 and 2018. Wild trees growing at 24 provenance field sites throughout the species’ range (left) compared to study trees growing at two common gardens (right). Central box plots show the the median date of bud break (white dot) and 1st and 3rd quartile values (black box bounds) while the surrounding curves show the distribution of individual bud break observations over the course of the season. Provenance sites served in 1990 as the original acorn sources for the common garden plantings.

2018 seasons, consistent phenological variation associated with individual provenance populations is readily identifiable in the common garden trees. Reaction norms plotted as mean bud break of trees from each provenance between the two garden locations show little difference in slope (lines are parallel) for the 26 provenance populations, indicating a consistent genetic effect of provenance site and similar degrees of plastic response across all of them (Fig. 3). This is confirmed by ANOVA models, which identify major components of phenological variation associated with both environment (two garden locations across 2 yr for four categories) ($F = 981.116$, $df = 3$, $P < < 0.0001$) and genetics (acorn provenance site) ($F = 29.314$, $df = 25$, $P < < 0.0001$) for the trees growing in the common gardens, as well as a significant, but very small interaction effect between environment and genetics ($F = 1.624$, $df = 75$, $P = 0.001$). Omega-squared (ω^2) calculated from the sums of squares in the ANOVA model shows 68.2% of the observed phenological variation at the common gardens can be associated with environment (garden location \times observation year) and 16.4% is associated with genetics (provenance location) while only about 1% of the observed variation is a $G \times E$ interaction (Table 1).

Using stepwise model selection on linear regressions with 36 weather variables for the 2017–2018 water years (monthly minimum temperature, monthly maximum temperature, and monthly pre-

TABLE 1. ANOVA PARTITION OF PHENOLOGICAL VARIATION AMONG OAK TREES GROWING AT TWO COMMON GARDEN LOCATIONS INTO COMPONENTS EXPLAINED BY PROVENANCE SITE (GENOTYPE, G), GARDEN LOCATION \times OBSERVATION YEAR (ENVIRONMENT, E), AND THE INTERACTION TERM ($G \times E$).

	Df	SS	F-statistic	P-value	ω^2
G	25	17203	29.314	2.136×10^{-82}	0.1642
E	3	69091	981.116	1.483×10^{-211}	0.6822
$G \times E$	75	2859	1.624	0.00143	0.0109
Residual	511	11995			0.1427

cipitation), we found that only a handful of these were needed to explain a large portion of the observed phenological variation. First, for bud break phenology observed at the provenance field sites, the full set of water year 2017–2018 weather variables explains 59.5% of the variation ($F = 27.49$ on 36 and 333 df, $P < < 0.0001$). Fall and spring monthly minimum temperatures have the most important effect in the models. November minimum temperature and March minimum temperature in the current water year alone explain 33.3% of the observed phenological variation at the provenance sites in 2017 and 2018 ($F = 93.23$ on 2 and 367 df, $P < < 0.0001$).

The single variable November minimum temperature of the current water year (i.e., prior calendar year) was particularly powerful in explaining observed bud break variation, across both provenance field sites and the two common gardens, with an R^2 of 42.0% across all sites in 2017 and 2018 and an effect size of -10.5 d for every degree-Celsius ($F = 713.7$ on 1 and 983 df, $P < < 0.0001$) (Figs. 4A and 5A). Multicollinearity between weather variables is common, though, and despite variance inflation factor (VIF) between November and March minimum temperature of <2.0 , indicating very low collinearity, parameter estimates of the effect size may still be unreliable. What is clear, though, is that higher minimum temperatures, especially in fall and spring months, advanced the timing of bud break at these sites.

The remaining phenological variation observed among trees at the common gardens, which cannot be explained by the current year weather they experienced, is partly explained by the climate of the acorn provenance sites sampled to plant them. The same 36 monthly temperature and precipitation variables, but as 30-yr climate mean from 1951–1980 rather than the current water year, in a combined model explain (adjusted R^2) 12.9% of the variation observed among the common garden trees ($F = 4.828$ on 25 and 589 df, $P < < 0.0001$). Multicollinearity was again a problem for these climate-based analyses, but maximum temperatures appear stronger in the climate effect. March maximum temperature for 1951–1980 was the single best predictor from the provenance populations of bud break in the common garden locations, although it explains only 2.4% of

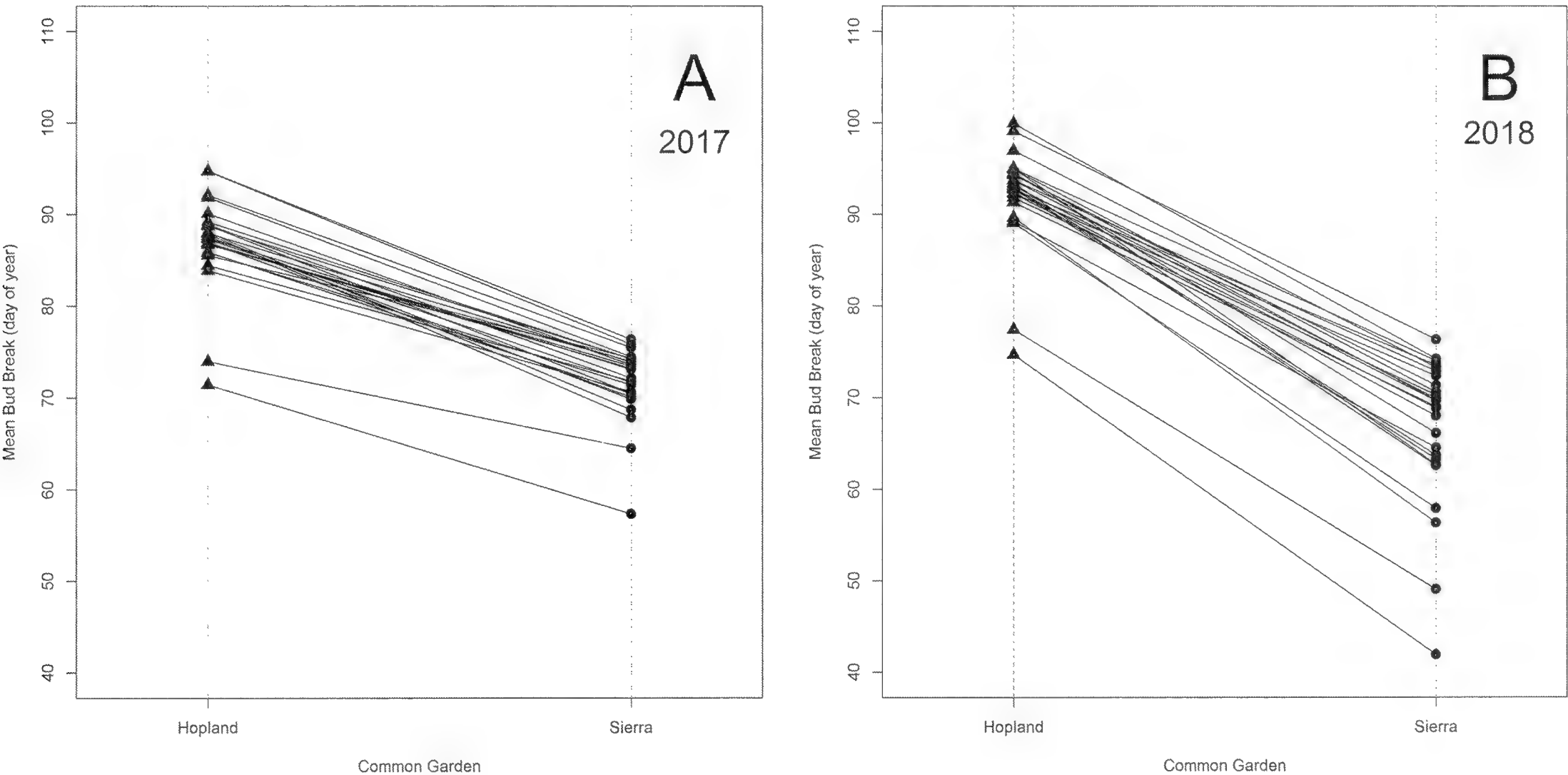


FIG. 3. Mean date of bud break in 2017 (A) and 2018 (B) for 26 provenance populations of *Quercus douglasii* growing at two common garden locations.

the observed variation, with a fairly consistent effect size of one day of bud break advancement for every degree-Celsius ($F = 12.57$ on 1 and 613 df, $P = 0.0004$) (Figs. 4B and 5B). In addition, 1951–1980 average March and September precipitation at the provenance sites were also identified as significant predictors, associated with later bud break in the common gardens. In combined models with March maximum temperature, all three climate variables

were able to explain a total of 4.8% of phenological variation in the common gardens.

Finally, these findings of genetic effects associated with provenance climate in the common gardens were applied back to both the garden and provenance field sites by including both the current water year weather variables (November and March minimum temperature) and the 1951–1980 30-yr mean of March maximum temperature and March

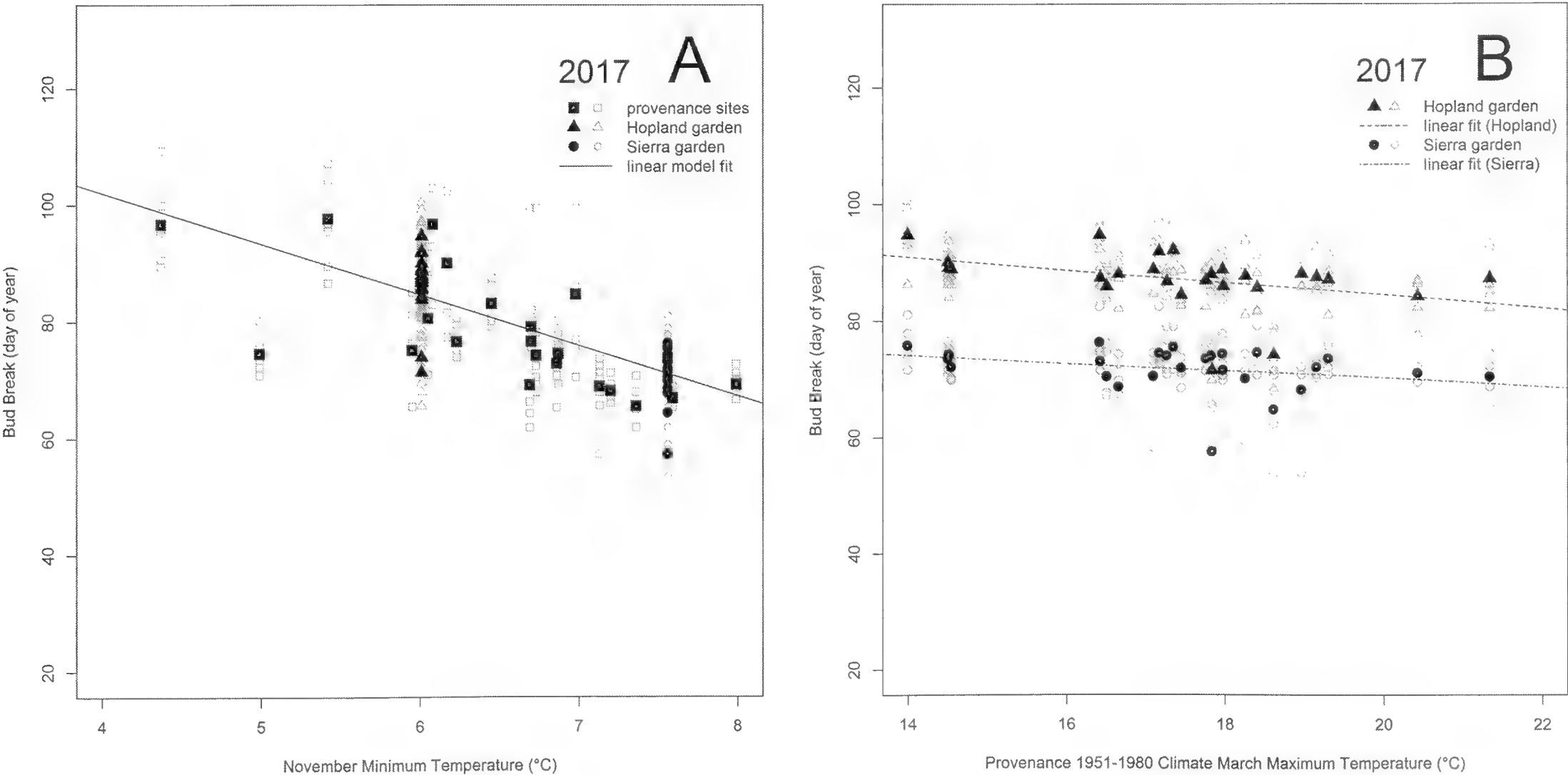


FIG. 4. 2017 bud break phenology for *Quercus douglasii*. (A) Bud break for all surveyed trees across two common gardens and 21 provenance field sites as a response to minimum temperature the previous November. (B) Bud break in the two common gardens (Hopland and Sierra Foothills) as a response to 1951–1980 climate March maximum temperature at the original acorn collection provenance sites. Open shapes in grey represent individual trees, while closed shapes are population means.

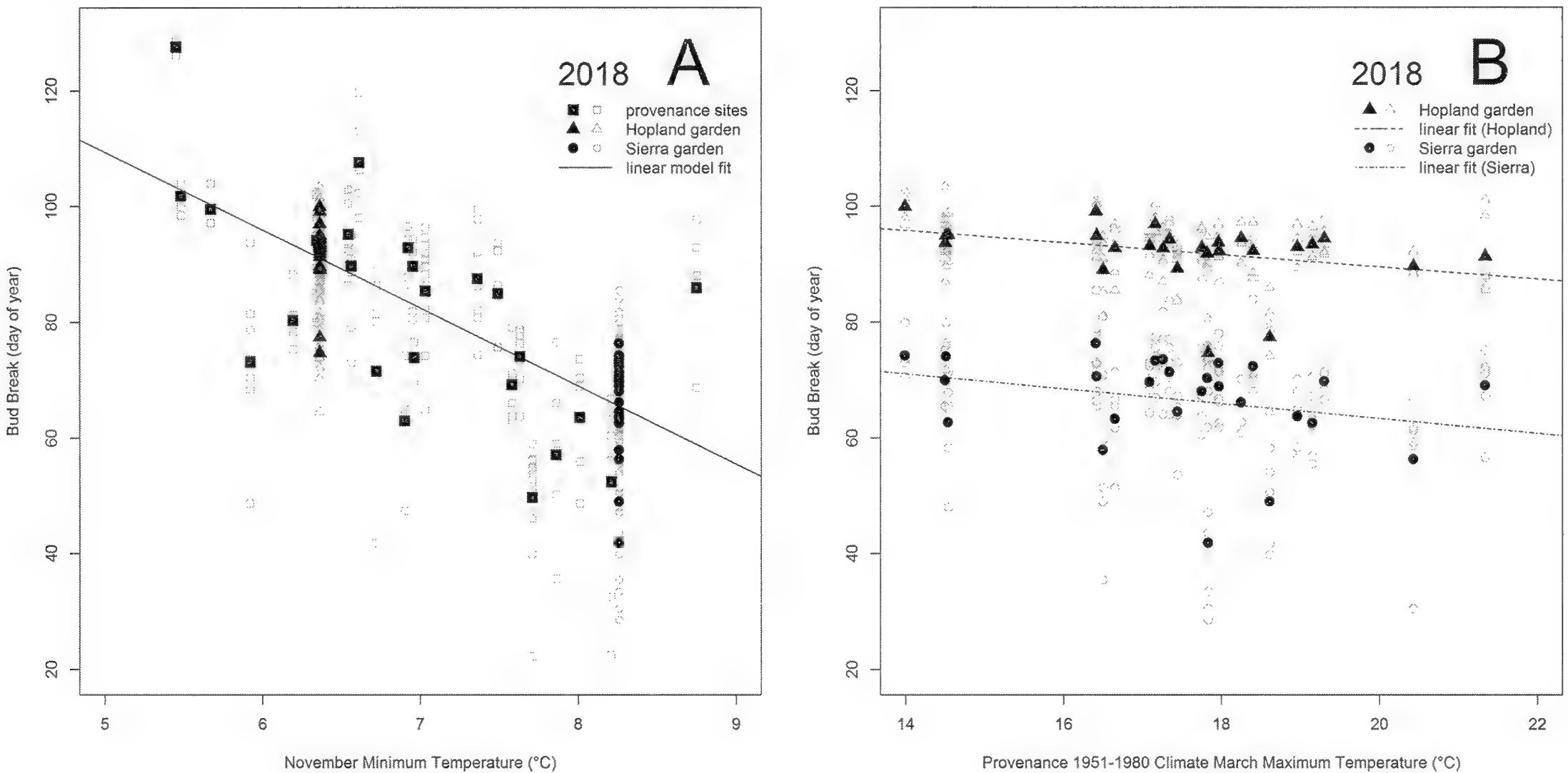


FIG. 5. 2018 bud break phenology for *Quercus douglasii*. (A) Bud break for all surveyed trees across two common gardens and 24 provenance field sites as a response to minimum temperature the previous November. (B) Bud break in the two common gardens (Hopland and Sierra Foothills) as a response to 1951–1980 climate March maximum temperature at the original acorn collection provenance sites. Open shapes in grey represent individual trees, while closed shapes are population means.

and September precipitation in a combined model (Table 2). Warmer November and March minimum temperatures experienced in the current water year advance bud break date by about 8 and 3 d per degree-Celsius, respectively, while warmer March maximum temperatures in the historical climate advance bud break by an additional day per degree-Celsius. These variables together explain 51.5% of the total observed phenological variation across all sites ($F = 209.6$ on 5 and 979 df, $P < 0.0001$). However, previous warnings about the effects multicollinearity among variables should still be applied here, despite VIF values < 2.0 .

DISCUSSION

Using an established common garden experiment planted from acorn collections across the range of *Quercus douglasii* (Blue Oak), with trees growing at two locations and more than 25 yr old at the time of the study, we found significant effects of both environment (common garden location and year)

and population level genetics (acorn provenance site) on the timing of spring bud break (Table 1). The genetic \times environmental interaction effect in the model is significant, but very weak, meaning that individuals responded uniformly based on their genetic background and the local environment. The remaining residual variation, similar in size to the provenance genetic effect, may be largely due to individual genetic variation among trees from the same provenance, though the effect of microsite soil and moisture variability within the common gardens is likely to be important as well. A large role for environmentally-driven phenotypic plasticity in phenology is expected (García-Mozo et al. 2002; Parmesan and Yohe 2003; Vitasse et al. 2009), while the significant genetic effect we found demonstrates a role of local evolutionary forces in shaping the phenological timing of blue oak as well.

Previous studies of oaks in both North America and Europe have reported high within population phenotypic variation across a range of traits; while a consistent local genetic effect can sometimes also be

TABLE 2. MULTIPLE REGRESSION RESULTS OF SELECTED CLIMATE AND WEATHER VARIABLES ON DATE OF BUD BREAK ACROSS BOTH GARDENS AND ALL PROVENANCE FIELD SITES FOR 2017 AND 2018. Historical climate (1951–1980) at the acorn provenance site represents a genetic component of variation in this model while the water year variables represent an environmental component. Adjusted $R^2 = 51.45\%$, $P < 0.0001$.

	Parameter Estimate	Standard Error	t-value	P-value
March max temp (1951–1980)	−1.0646	0.2291	−5.646	3.845×10^{-6}
March precip (1951–1980)	0.0586	0.0147	3.868	1.169×10^{-4}
September precip (1951–1980)	0.4700	0.0754	6.234	6.744×10^{-10}
November min temp (water year)	−8.0369	0.4142	−18.963	1.461×10^{-68}
March min temp (water year)	−2.7827	0.2653	−10.525	1.283×10^{-24}

identified, its importance varies widely (Derory et al. 2010; Ramírez-Valiente et al. 2010; Cavender-Bares and Ramírez-Valiente 2017). Previous results even from this same common garden experiment illustrate how the magnitude of genetic effects may depend on what aspect of phenotype is studied. Shoot growth over the first 4 yr in the common gardens was found to be strongly influenced by provenance site (McBride et al. 1997), but more recent ecophysiological work on the same trees found no variation among provenances in their susceptibility to vascular embolism (Skelton et al. 2019).

Comparison with the study of Wright et al. (2021) published in this special issue is especially interesting. Using a common garden design similar to ours, they found significant genetic effects of provenance climate on the spring phenology in Valley Oak (*Quercus lobata*). Their analysis focused on bioclim summary variables (rather than monthly climate) and did not include bioclim_8, which is most similar to the March maximum temperature that we identified as a significant climate predictor. However, both maximum temperature of the warmest month (bioclim_5) and elevation, which is strongly associated with spring temperature, had effects consistent with our findings for Blue Oaks. They also found that a climate history of precipitation in both the wet season (bioclim_13) and dry season (bioclim_14) delayed phenology, which is again consistent with our findings for the genetic effects associated with March and September precipitation.

In our study, the environmental component of phenological variation across all sites could largely be explained by minimum temperatures in the months preceding bud break, especially November and March (Figs. 4A and 5A), while the genetic component, as revealed in common garden conditions, was in part associated with climate history at the provenance locations. A composite climate effect in regression models with only common garden trees explained 12.9% of the bud break variation, which compares well with the 16.4% explained by provenance site as a categorical variable in ANOVA. No single monthly climate variable was found to explain a major portion of this genetic effect, but March maximum temperature was consistently significant, though alone it only explained a small amount of the observed phenological variation (Figs. 4B and 5B).

The magnitude of the effect of temperature forcing on spring phenology we report in these oaks (Table 2) is similar to figures that have previously been reported for oaks in the Mediterranean region (Sanz-Pérez et al. 2009) and northern European temperate forest trees, including *Quercus robur* L. (Roberts et al. 2015). Between 5 and 10 d of phenological advance per degree-Celsius of temperature increase is commonly found. However, we must note again that, despite VIF in all our models < 2.0 (indicating very low multicollinearity), parameter estimates remained sensitive to exactly which monthly temperature and precipitation variables we included together

in a model. As a result, we do not believe that the parameter estimates for the effects of November or March minimum temperature in our models and reported here should be considered definitive. Instead, the responses we highlight involving these particular months point to a more general importance of low temperature, in both fall and spring, as an environmental influence on phenological timing in Blue Oaks. A summary statistic like monthly minimum temperature is ultimately not likely to be the direct environmental factor influencing the timing of phenology. Similar to geographic proxies that are often used (e.g., elevation or latitude), monthly minimum temperatures are correlated with mechanistically proximate factors. Variable selection and parameter estimates in models will depend on the strength and interactions involved in those correlations.

We did not find that winter cold temperatures advanced the timing of bud break (i.e. chilling hours). In fact, the relationship between winter temperature and spring phenology was negative, so that colder temperatures in winter delayed bud break just as they did in spring. This finding agrees with previously reported results in European oaks from both Mediterranean and continental climates (García-Mozo et al. 2002; Roberts et al. 2015; Wilkinson et al. 2017). Oaks do not seem to require chilling-hour accumulation during dormancy to set buds as seen for some other temperate forest trees (e.g., birch or hawthorn). Instead, temperature has a uniform forcing effect, with warmer temperature advancing bud break throughout the entire dormant season.

Our results suggest that the debate over assisted gene flow versus using local seed sources should not in fact be a major concern in the case of Blue Oaks, at least when considering spring phenology with its important implications for pollination success, freeze-damage, productivity, and herbivory. While a significant signal of local adaptation in phenological timing was observed, there was a high variation among trees collected from the same provenance site, often encompassing almost the total variation observed across all provenance sites (Figs. 4B and 5B, open shapes). Thus, while climate-informed assisted gene flow seems unlikely to disrupt patterns of local adaptation, it also may not be particularly beneficial in the case of Blue Oaks. Local populations of Blue Oak have been shown to harbor large amounts of genetic variation (Rice et al. 1997; Papper unpublished data) and this within-population genetic variation, together with the microsite variation within both gardens and provenance sites, likely explains much of the residual phenological variation that was not explained in this study. If the local phenological variation within sites is comparable to variation across the species' entire range, as it seems to be, that existing local variation already provides the potential to allow populations to track changing climate and assisted gene flow would add little to

their adaptive potential. This agrees with results found in other oaks, for which within population variation has been suggested as important for seedling establishment (González-Rodríguez et al. 2012). The critical question is whether cycles of seedling recruitment, maturation, and mortality will be able to keep pace with the rate of changing climate. This process can be aided by restoration efforts, with or without involving assisted gene flow. For restoration purposes, the most important recommendation may simply be to plant more acorns, in order to better capture the adaptive potential of whatever population is chosen as a seed source. The advantage of assisted gene flow should be seen as only a somewhat increased probability of planting well-adapted genotypes, in which case fewer acorns may be necessary. This could warrant consideration in terms of cost- and labor-saving in restoration projects.

By building on the legacy of the two established common garden plantings, combined with new field work tracking phenological activity and high-resolution monthly temperature and precipitation model data from PRISM/BCM, we have been able to tease out a significant component of genetic phenological adaptation in Blue Oaks and associate it with the temperature and precipitation history of the provenance sites that provided acorns for the common garden plantings. This study illustrates a powerful combination of data sources with the potential to be replicated in many other cases. A rich legacy of mature common garden plantings, involving a variety of woody species, can be found languishing, largely unknown, across California and outside the state. The availability of modeled climate data has the potential to make these gardens very valuable if the seed source locations are or can be established, as they were in this case. Broad efforts should be made to document and catalog historical common garden plantings such as these and collate whatever data is available about them, so that they can be utilized in a new generation of research informing climate adaptation strategies.

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BUDBURST TIMING OF VALLEY OAKS AT HASTINGS RESERVATION, CENTRAL COASTAL CALIFORNIA

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ABSTRACT

We studied the timing of budburst of valley oak (*Quercus lobata* Née) at Hastings Reservation, central coastal California. Similar to other taxa, budburst was advanced by warmer temperatures. Over the 30-year study period, however, there were no significant trends in either air temperature or the timing of budburst, except during the 2014–2016 drought, during which the earliest budburst dates were advanced. Several individual tree characteristics correlated with budburst timing, including access to ground water, soil available phosphorus, and elevation, the effects of which were in turn correlated with winter microclimatic conditions of individual trees. Budburst timing was significantly related to both subsequent acorn production and radial growth; trees leafing out on or near the population mean for the year experienced greater radial growth and produced larger acorn crops than trees leafing out earlier or later than the mean. Differences in acorn production were due to both differences in phenology among trees and plasticity in the phenology of individual trees across years, while differences in radial growth were primarily due to plasticity in individual tree phenology. Valley oak phenology exhibits considerable variability; the extent to which this plasticity will help this keystone California species adapt to future climate change remains to be seen.

Key Words: budburst, climate change, phenology, *Quercus lobata*, valley oak.

The connection of vegetation phenology to meteorological conditions has been recognized since at least the first half of the 18th century, when the French naturalist Reaumur (1735) proposed the method of cumulative thermal summation as a means of predicting the onset of budburst in temperate trees. Since then, the study of phenology has become an industry unto itself due to its role in reconstructing and predicting primary productivity in this era of global climate change (Chmielewski and Rötzer 2001; Badeck et al. 2004; Menzel et al. 2006, Piao et al. 2019). Nonetheless, many questions remain. In particular, although a close relationship between environmental conditions—primarily temperature—and budburst is well established in many taxa, other factors influencing phenology are poorly studied, despite evidence demonstrating considerable variability in phenological landscapes over small spatial scales both within and between species (Denéchère et al. 2021). Studies also indicate that genetics (Faticov et al. 2020; Papper and Ackerly 2021; Wright et al. 2021), elevation and soil type (Cole and Sheldon

2017), and even anthropogenic light pollution (French-Constant et al. 2016) can affect tree phenology. Analysis of the factors influencing phenological differences both within populations and across years has the potential to improve our understanding of a wide range of ecological factors affecting plant populations, including seed production, disease susceptibility, and insect damage, as well as the likely effects of future climate change (Koenig et al. 2015; Pearse et al. 2015a; Bogdziewicz et al. 2017; Faticov et al. 2020).

We studied budburst phenology in a population of valley oak (*Quercus lobata* Née) over a 30-year period, 1991–2020. Prior work on this population has focused on the relationships among budburst timing, phenological synchrony, and acorn production (Koenig et al. 2012, 2015), and the relationship between budburst timing and herbivore damage (Pearse et al. 2015a). Here we look specifically at within-population and among-year variability in budburst. We address three issues: (1) variability in annual budburst across years and among individuals

within the population; (2) factors correlating with budburst phenology; and (3) the relationship between budburst and subsequent acorn production and radial growth, including the relative importance of within- vs. between-tree effects.

METHODS

Species and Study Site

Quercus lobata is a “white” oak in the subgenus *Quercus* (Pearse and Hipp 2009). It is winter deciduous, matures acorns in one year, and, like other members of this genus, wind-pollinated and an obligate outcrosser. Its range, entirely within California, encompasses foothill regions ringing the Central Valley below ~1,800 m in elevation (Griffin and Critchfield 1972). Throughout this range, stands typically consist of relatively few large trees (White 1966; Bolsinger 1988). Our study site, Hastings Natural History Reservation (36°12’N, 121°33’W), is in the outer coast range of Monterey County in central California. This locality consists of typical coastal oak savanna dominated by valley, blue (*Q. douglasii* Hook & Arn.), and coast live (*Q. agrifolia* Née) oaks interspersed within a mixed deciduous forest and grassland matrix (Griffin 1974). The climate is Mediterranean with warm, dry summers and cool, wet winters. Based on 30 years of records taken between 1990 and 2019 at the Reserve headquarters, mean annual rainfall is 534 mm and mean annual temperature is 14.0°C.

Individual Tree Variables

Budburst for 25 individual *Q. lobata* was measured each year from 1991–1996, inclusive. The survey was restarted and expanded in 2003 to encompass 86 trees (including the original 25), chosen as part of a study of acorn production. Thus, the data analyzed here covered the 30 years (24 of which were surveyed) of 1991–1996 and 2003–2020. Diameter at breast height (dbh) and elevation were recorded for each tree.

Each spring we surveyed trees weekly for budburst starting between mid-January and 1 March, depending on the year. Budburst was used as a proxy for flowering and pollen production, which follow budburst by an average of 12.3 days (Koenig et al. 2012). Analyses were based on the day-of-year when a tree was determined to have undergone budburst, defined as the first date on which at least 5% of the tree had leafed out and turned green. Occasionally a tree leafed out prior to the first survey of the year. If 25-75% of the tree had leafed out prior to the first survey, we estimated budburst as having occurred 7 days prior to the first survey date (2.7% of tree-years). If >75% of the tree had leafed out as of the first survey, budburst was estimated to have been 14 days prior to the first survey date (0.9% of tree-years).

For each of the 86 trees we measured water availability, soil available nitrogen (N), soil available phosphorus (P), and winter microclimatic temperature. Water availability was measured by predawn xylem water potential in September 1991 using a pressure bomb (Knops and Koenig 1994). Although values vary from year to year depending on rainfall, differences in xylem water potential values are concordant among trees (Knops and Koenig 2000). Thus, although only measured once, these values provide a good index of overall differences in water stress among trees. Soil available N and P were estimated using four ion-exchange resin bags placed 5–10 cm in the soil underneath each tree from October 1992 to April 1993. Resin bags were subsequently analyzed for NO₃, NH₄, and PO₄ with values expressed and mg/l effluent (Knops and Koenig 1997).

Acorn production was estimated each year in September using visual surveys. Two observers scanned different parts of the canopy of each tree and counted as many acorns as they could in 15 s (Koenig et al. 1994, 1996). Values were added together and *ln*-transformed to reduce non-normality. Radial growth was measured using dendrometers (Cattellino et al. 1986) established in 1994 and subsequently measured annually at the time of the autumn acorn survey. Annual dendrometer growth values were scaled within individual trees to a mean of 0 and standard deviation of 1.

Environmental Variables

Daily temperature (maximum and minimum) and rainfall were obtained from the Hastings Reserve weather station located within 3.5 km of all trees. As rainfall in the Mediterranean climate of the study area falls almost entirely between early November and late March, we summed rainfall for each year from 1 November to the mean budburst date of the survey trees; we refer to this variable as winter rainfall. Mean daily temperature in our study site is rarely below 0°C; consequently, using a cumulative degree day model was not feasible. Instead, we used an iterative linear predictor (Bush and Mosteller 1955) to quantify temperature, which mitigates problems associated with measuring values over a fixed period (Gienapp et al. 2005). The linear predictor weighted values according to the formula:

$$\lambda(t) = \text{daily temperature}(t) * \lambda\alpha + \lambda(t + 1) * \alpha,$$

where $\lambda(t)$ is the weighted mean temperature on day t and α the weighting factor. We started calculations on 1 December of the prior year and ended at the mean (for the population analyses) or individual tree budburst date. Calculations started on 1 December because valley oaks often retain acorns and leaves into November, but only rarely longer (Koenig et al. 2014).

λ weights each day’s temperature by α^n , where n was the number of days prior to budburst. Prelim-

inary analyses comparing daily maximum, daily minimum, and daily mean ($[\text{maximum} + \text{minimum}] / 2$) temperature and values of α between 0.01 and 1.0 ($\alpha = 1.0$ is tantamount to averaging mean daily values) indicated that the best predictor of budburst was using mean daily temperature and $\alpha = 0.98$, which predicted budburst significantly better than averaging mean daily temperatures (ANOVA comparing $\alpha = 0.98$ and $\alpha = 1$; $\chi^2 = 24.3$; $P < 0.001$). Thus, each day's mean daily temperature prior to budburst was weighted 98% as heavily as the subsequent day. As an example, the λ -weighting factor 30 days prior to budburst was $0.98^{30} = 0.545$. Statistical analyses used the weighted mean temperature values, but (unweighted) mean temperature values were used in figures to simplify interpretation of observed relationships.

Winter microclimate was measured using small, automated temperature recorders (iButtons; Maxim Integrated Products, Sunnyvale, CA) located on the north side of each tree ~ 1.5 m above ground (Koenig et al. 2015). iButtons were programmed to record temperatures at 4-hr intervals each day starting at 04:00 hours. For the analyses performed here, we calculated the mean of the daily maximum and minimum temperatures for each tree between 1 December and 31 March for the years 2004–2019; the 08:00 temperature reading was not included due to logistic issues. We then averaged mean values across all years to yield an overall index of the winter microclimate of each tree. Preliminary analyses indicated that correlations with mean minimum values were much stronger than with mean maximum values, and thus we used only mean minimum values (referred to as winter microclimate) to analyze relationships between trees experiencing different microclimates and the other tree characteristics measured.

Data Analysis

Analyses were conducted in R 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria) using Cox proportional hazards models (procedures *coxph* and *coxme* in package *coxme*) and Pearson correlation coefficients. For the Cox proportional hazards models, we list the exponential of the regression coefficients for the fixed effects ($\exp(\beta)$). $\exp(\beta)$ is the ratio of the hazards between two individuals whose values differ by one unit of the fixed factor when all other covariates are held constant. Thus, positive values indicate that the variable positively affects the response variable, while negative values indicate the reverse. In order to render effect sizes comparable within models, all variables were scaled to a mean of 0 and standard deviation of 1 prior to analysis.

Cox proportional hazards models in which the response variable was the mean, earliest, or latest budburst date of the year included weighted mean temperature, winter rainfall, and time (year) as fixed

factors. These models tested the effects of temperature and rainfall on budburst of the population and whether there was a temporal trend in budburst timing over the length of the study. For individual trees, we calculated Pearson correlation coefficients between variables. We did this because some variables were measured only once for each tree (xylem water potential and soil nutrients) or were characteristics of the trees that stayed the same (elevation) or were concordant (dbh) during the study, and because correlations between microclimate and the tree variables helped interpret their relationships with budburst date. For these individual tree analyses, we used mean budburst date of trees calculated by averaging the day-of-year budburst date for each individual across the years when all trees were surveyed (2003–2020).

To test the effects of budburst date on radial growth and the subsequent acorn crop, we calculated budburst for each tree relative to the annual mean of all trees ($[\text{budburst of tree } i \text{ in year } x] - [\text{mean budburst date of all trees in year } x]$) and used this value (both linear and quadratic) as fixed factors in mixed-effects models (procedure *lmer* in package *lmerTest*) in which the acorn crop or radial growth of the tree in year x was the response variable and *tree ID* was a random effect.

Finally, we performed analyses distinguishing whether the observed effects of phenology on growth and reproduction were due to differences among trees (between-tree effects) or plasticity in the phenology of individual trees (within-tree effects). For each tree, we calculated the mean budburst date across all years and the difference between a tree's observed budburst date in a particular year and its mean budburst date (centered budburst date). Including these two variables in a linear regression model as fixed effects distinguishes within-tree effects (centered budburst date) from between-tree effects (mean budburst date) (van de Pol and Wright 2009).

RESULTS

Annual Budburst Timing

A summary of the earliest, latest, and mean budburst dates illustrates the variability observed among years, with mean budburst ranging from Day 48 (18 February 2015) to Day 93 (3 April 2012) (Fig. 1a). The earliest budburst date detected during the study was Day 14 (14 January 2015). Mean (unweighted) temperature from 1 December to the mean budburst date and winter rainfall were also highly variable (Fig. 1b). Cox proportional hazards models testing the effects of weighted mean temperature, rainfall, and year on budburst dates indicated that the mean, earliest, and latest budburst dates all were significantly advanced when temperatures were warmer (Table 1; Fig. 2a). Budburst date was generally delayed in wetter years, but the effect of rainfall was not significant (Table 1; Fig. 2b). Over

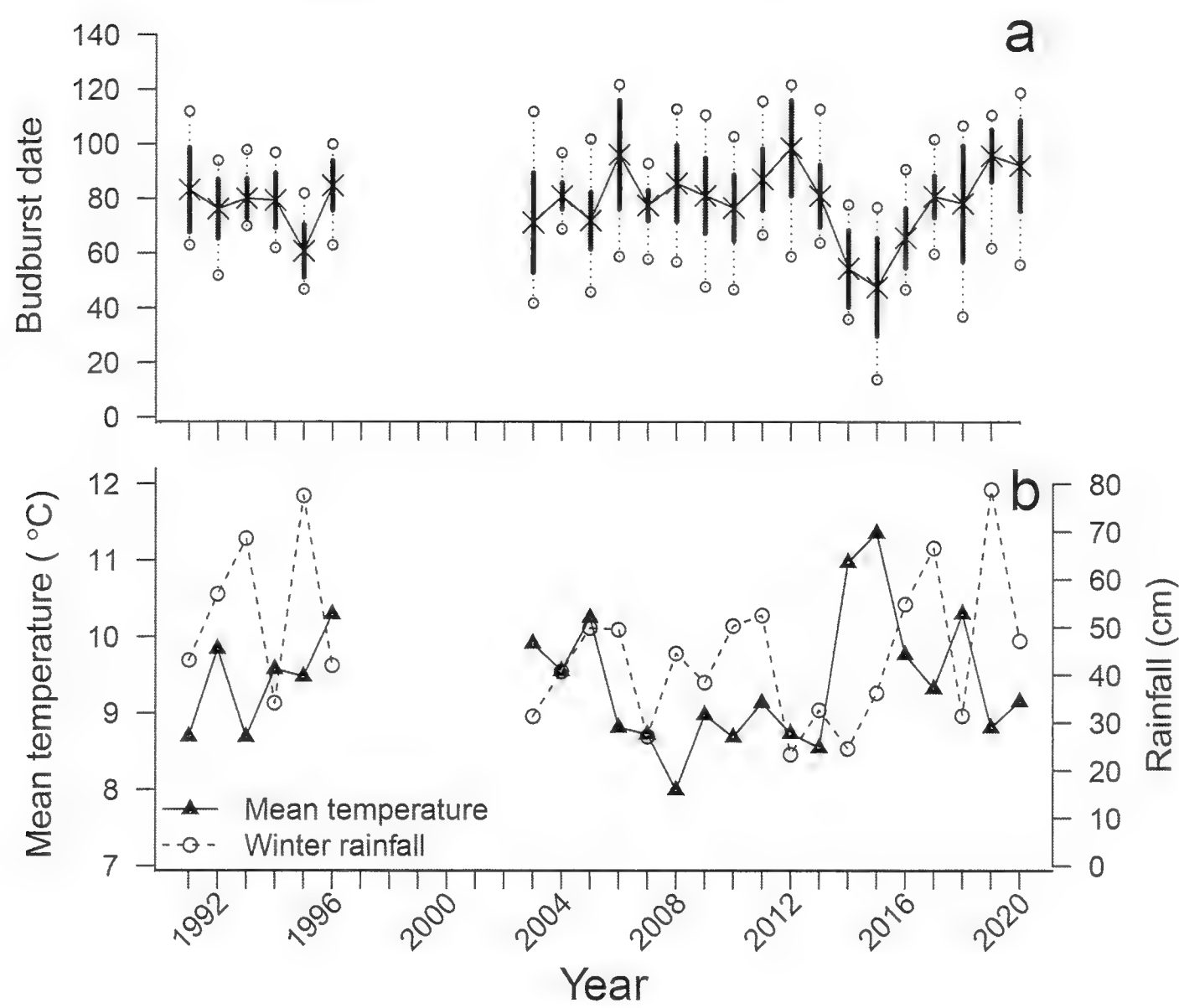


FIG 1. (a) Summary of annual mean budburst dates for valley oaks at Hastings Reservation, 1991–1996 and 2003–2020. Plotted are the mean (marked by X), standard deviation (thick lines), and earliest and latest (dashed lines) of budburst dates, 1991–1996 and 2003–2020. (b) Mean temperature (1 December of the prior year to mean budburst date; plotted values unweighted) and seasonal rainfall (1 October of the prior year to 30 March).

the course of the study, there was no significant trend in the mean or the latest budburst date as indicated by the ‘year’ term. There was, however, a significant positive trend in the earliest budburst date (Table 1).

Budburst Timing of Individual Trees

Correlations of mean budburst date and winter microclimate with water availability, soil nutrients, elevation, and dbh are summarized in Table 2. Trees growing in sites with warmer winter microclimates experienced earlier budburst (Fig. 3a). Budburst was also earlier among trees that had lower water availability and higher soil P (Fig. 3b); there were no significant relationships with soil N. Trees growing in warmer winter microclimate sites had less water availability and higher soil P (Fig. 3c).

Relationship Between Budburst and Fitness

Mixed-effects models revealed significant non-linear effects of budburst on both a tree’s subsequent acorn crop and radial growth (Table 3, top). In both cases, trees undergoing budburst relatively early and relatively late produced smaller acorn crops and experienced less radial growth than trees whose phenology matched the mean of the population for the year (Fig. 4).

In models including both between-tree (mean budburst date) and within-tree (centered budburst date) effects, both contributed significantly to the effects of phenology on the subsequent acorn crop (Table 3, bottom). Only the mean within-tree effect (centered budburst date) exhibited a significant relationship with radial growth.

TABLE 1. RESULTS OF COX PROPORTIONAL HAZARDS MODELS OF ANNUAL BUDBURST DATES OF VALLEY OAK AT HASTINGS RESERVATION, 1991–1996 AND 2003–2020. Log-likelihood ratios and regression coefficients for three models in which the mean, earliest, and latest budburst date of the population were the response variables. All models included the mean weighted temperature, rainfall, and year as fixed effects, scaled (mean = 0, standard deviation = 1) prior to analysis. A regression coefficient larger than 1 means that a variable had a positive effect on the hazard; that is, it increased the probability of budburst. * P < 0.05; *** P < 0.001; other P > 0.05.

Response variable (budburst date)	Fixed effects			Overall likelihood ratio (df = 2)	Overall P-value
	Weighted mean temperature	Winter rainfall	Year		
Mean	10.82***	0.95	1.02	34.86	<0.001
Earliest	2.17*	0.69	1.72*	11.95	0.008
Latest	8.45***	1.55	0.73	32.74	<0.001

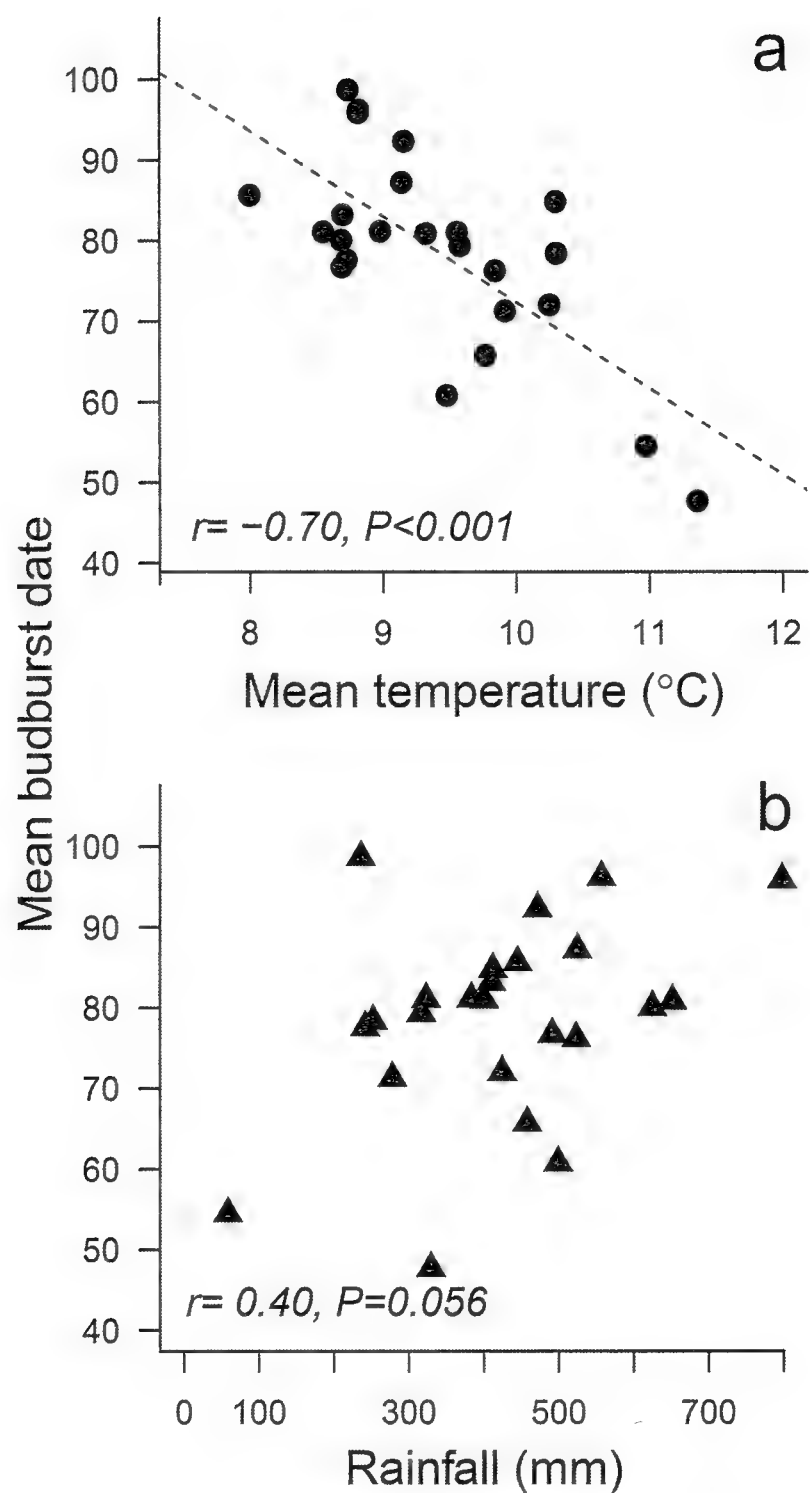


FIG. 2. (a) Mean budburst date of valley oak at Hastings Reservation (day-of-year) vs. mean temperature (1 December to mean budburst date), 1991–1996 and 2003–2020. (b) Mean budburst date of valley oak vs. winter rainfall (1 November to mean budburst date). In both cases, each dot represents a year (n = 24 years).

DISCUSSION

Budburst is a relatively easily measured indicator of climate effects (Cleland et al. 2007). In order to interpret the role that climate plays in affecting budburst, however, it is important that other factors influencing phenology are recognized. Although a variety of genetic, environmental, and anthropogenic factors have been found to affect phenology in various tree species, few studies have examined the effects of multiple factors on budburst. Phenology can also have important effects on the fitness of plants, affecting the timing of species interactions (Yang and Ruidolf 2010), resource acquisition (Nord and Lynch 2009), insect herbivory (Pearse et al. 2015a, 2015b), and reproduction (Galen and Stanton 1991; Koenig et al. 2012). The role of phenology as both an indicator of change and as a driver of fitness has made it the focus of a wide range of ecological studies.

We measured the timing of budburst over three decades on a population of 86 *Quercus lobata* for which we had data on temperature, water availability, soil nutrients, size, growth, and reproduction. As found in numerous prior studies, temperature had a

TABLE 2. PEARSON CORRELATION COEFFICIENTS BETWEEN MEAN BUDBURST DATE (LEFT) AND WINTER MICROCLIMATE (RIGHT) AND INDIVIDUAL TREE VARIABLES OF VALLEY OAK AT HASTINGS RESERVATION, 1991–1996 AND 2003–2020. * P < 0.05; ** P < 0.01; *** P < 0.001; other P > 0.05, n = 86 trees.

Variable	Mean budburst date	Winter microclimate
Mean budburst date	—	−0.55***
Predawn xylem water potential	0.41***	−0.52***
Soil available P	−0.24*	0.34**
Soil available N	−0.02	−0.14
Elevation	−0.37***	0.70***
Diameter at breast height	−0.13	0.10

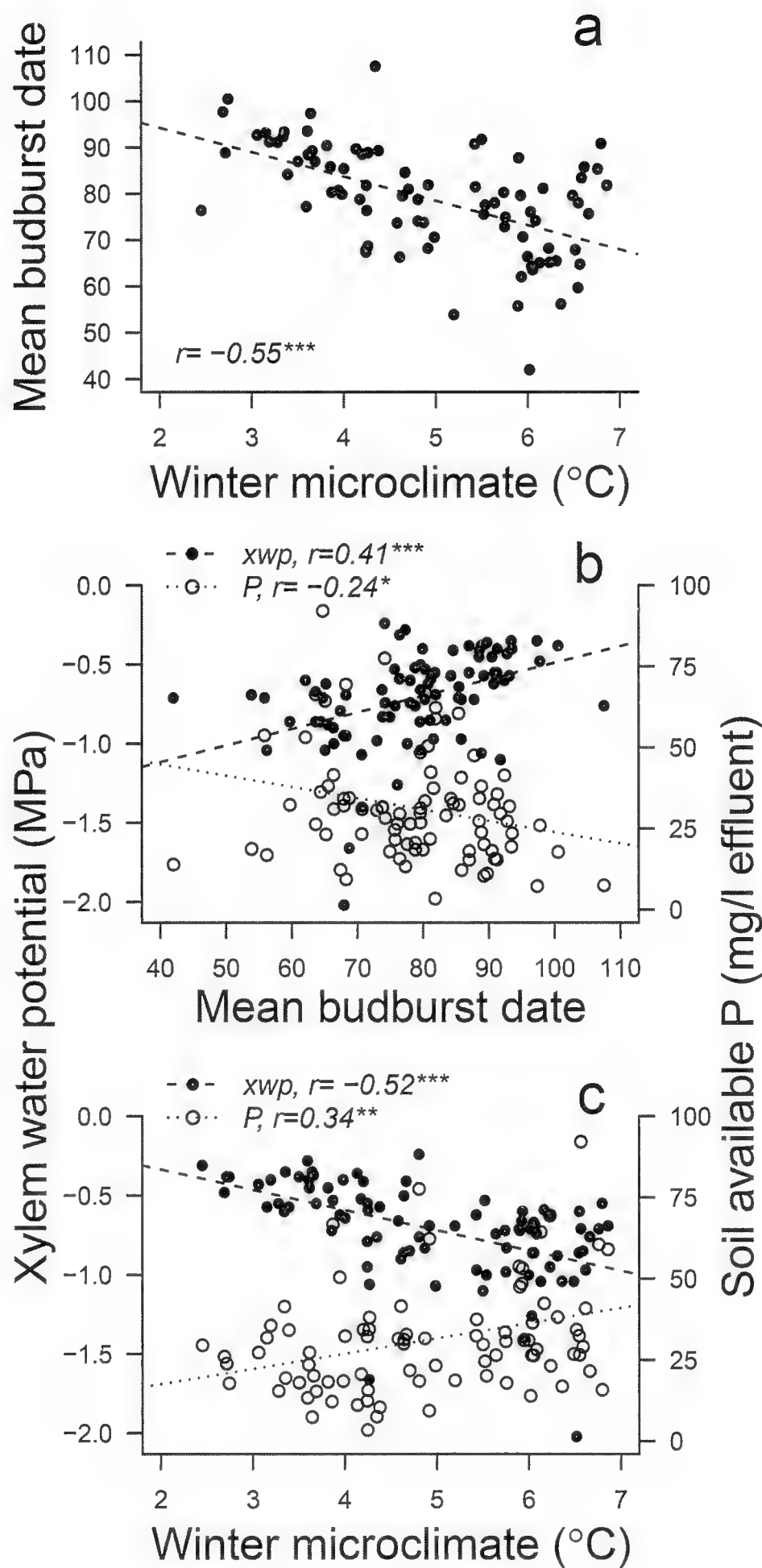


FIG. 3. Scattergrams of variables measured for 86 individual valley oaks at Hastings Reservation. (a) Winter microclimate (2004–2019) vs. mean budburst date (2003–2020). (b) Mean budburst date vs. xylem water potential (xwp) and soil available phosphorus. (c) Winter microclimate vs. xylem water potential and soil available phosphorus. * P < 0.05; ** P < 0.01; *** P < 0.001.

TABLE 3. RESULTS OF MODELS TESTING THE EFFECTS OF RELATIVE BUDBURST DATE (LINEAR AND SQUARED TERMS) (TOP) AND WITHIN- VS. BETWEEN-TREE EFFECTS OF PHENOLOGY (BOTTOM) ON REPRODUCTION AND GROWTH OF VALLEY OAK AT HASTINGS RESERVATION, 1991–1996 AND 2003–2020. Acorn crop was *ln*-transformed; radial growth was standardized within trees. *Tree ID* included as a random effect in the models testing for the effects on reproduction and growth. Effect sizes multiplied by 100. ** $P < 0.01$; *** $P < 0.001$; other $P > 0.05$.

Fixed effects	Response variable	
	Acorn crop	Radial growth
<i>Effects on reproduction and growth</i>		
Budburst date	-0.065 ± 0.393	$-0.709 \pm 0.180^{***}$
(Budburst date) ²	$-0.111 \pm 0.015^{***}$	$-0.050 \pm 0.009^{***}$
<i>Within-tree vs. between-tree effects</i>		
Mean budburst date	$15.111 \pm 4.658^{**}$	2.184 ± 2.614
(Mean budburst date) ²	$-0.088 \pm 0.030^{**}$	-0.016 ± 0.017
Centered budburst date	$0.730 \pm 0.281^{**}$	0.007 ± 0.158
(Centered budburst date) ²	-0.019 ± 0.012	$-0.054 \pm 0.007^{***}$

strong effect on budburst, with warm temperatures advancing the budburst date of the population (Fig. 2a) and of individual trees (Fig. 3a). Using the weighted mean temperature variable, each experienced unit of $\lambda(t)$ increased the probability of population budburst by over an order of magnitude (Table 1). Rainfall was not significant in a multivariate analysis including temperature, although the correlation between annual rainfall and mean budburst date was positive and nearly significant (Fig.

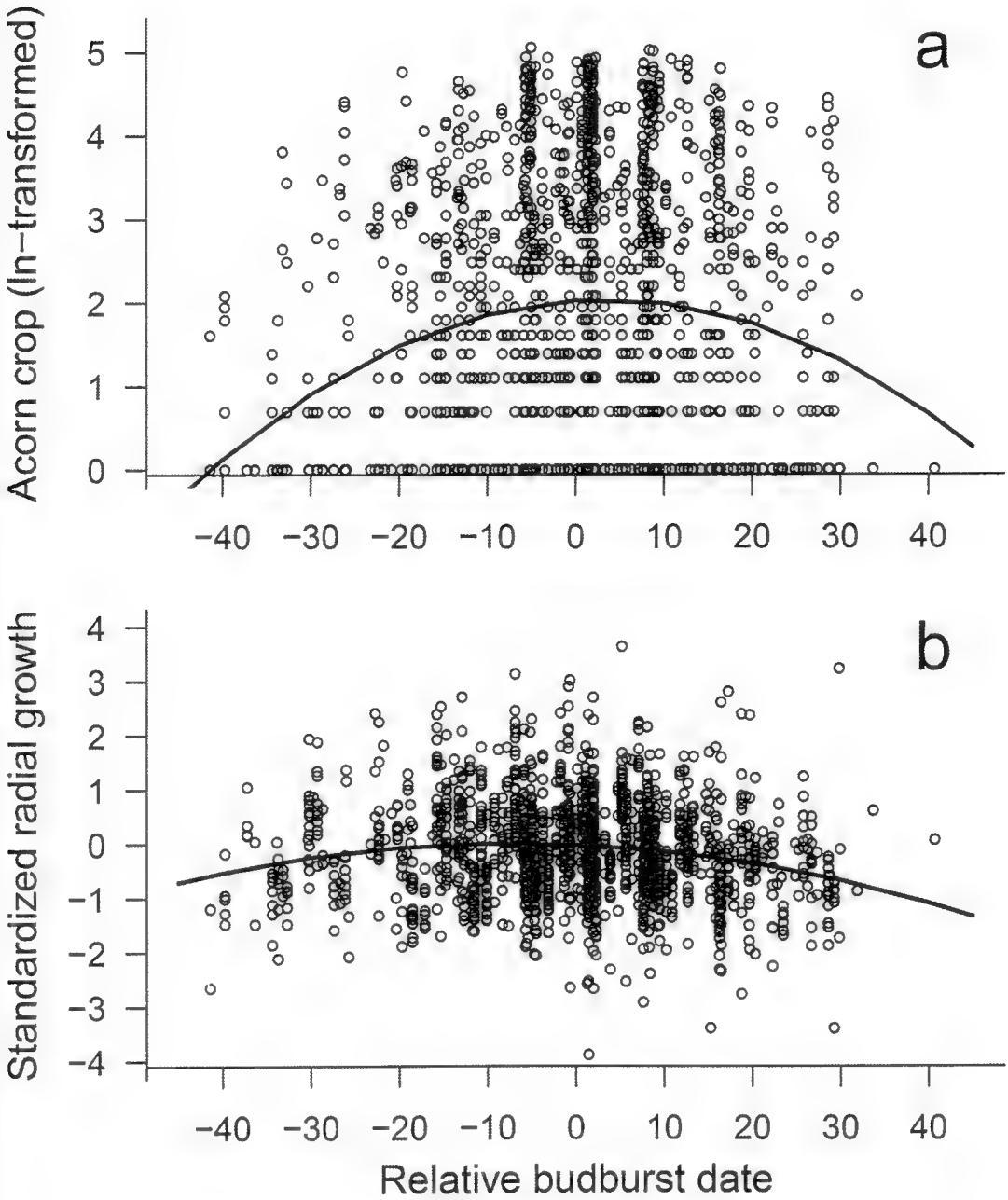


FIG. 4. (a) Scattergram of the (*ln*-transformed) acorn crop vs. the prior spring’s relative budburst date (budburst relative to the mean budburst date of valley oaks that year). (b) Scattergram of standardized radial growth vs. the prior spring’s relative budburst date. Both lines plotted using predicted values from regressions including both linear (budburst date) and quadratic terms (budburst date squared). For statistical tests, see Table 3.

2b). This most likely reflects rainfall being inversely correlated with maximum temperatures: over the same 24 years for which phenology was surveyed, there was a significant inverse correlation between mean maximum temperature and rainfall during the winter (November through March) (Pearson correlation $r = -0.51$; $P = 0.01$; $n = 24$ years). Thus, wetter conditions correlate with cooler, more moderate temperatures at the study site and elsewhere in the Mediterranean regions of California, making the effects of temperature and rainfall on phenology difficult to distinguish (Gerst et al. 2017; Armstrong-Herniman and Greenwood 2021).

Over the time period of the study there was little overall trend in the timing of budburst, with only the earliest budburst date advancing significantly (Table 1). Examination of Fig. 1a suggests that this was most likely due to the extreme drought years of 2014–2016 (Griffin and Anchukaitis 2014; Luo et al. 2017), during which winter rainfall was low and temperatures high.

Overall, annual mean temperatures at the study site did not increase significantly during the years of the survey (Pearson correlations between year and annual mean maximum, annual mean minimum, and annual mean average temperatures = 0.20 [$P = 0.37$], 0.24 [$P = 0.27$], and 0.36 [$P = 0.09$], respectively). Models, however, suggest that climate change is likely to have significant effects on California oak distributions in the future, including valley oak, primarily due to warming temperatures rather than changes in precipitation (Kueppers et al. 2005; Loarie et al. 2008). Thus, although we detected no clear trend over the course of this study, valley oak phenology will potentially advance in the future. Although valley oaks are winter deciduous, the relatively moderate climate occasionally allows acorns of this species to remain on the trees throughout the winter (Koenig et al. 2014). In addition, the length of time that valley oaks are without leaves at our study site is as short as 3 months (mid-November/early December through mid-February/early March). We did not record timing of leaf senescence in this study, but both

budburst and senescence will potentially be influenced by climate change (Firmat et al. 2017; Zani et al. 2020), affecting the length of the growing season and fitness via seed production (Journé et al. 2021) and susceptibility to insect herbivory the following spring (Karban 2007).

Other than temperature, we detected several variables correlating with budburst of individual trees, including water availability, soil P, and elevation (Table 2). In general, valley oaks avoid drought by tapping into ground water on alluvial terraces (Knops and Koenig 1994). Nonetheless, trees with relatively poor access to ground water, and thus under greater water stress, leafed out significantly earlier than trees with greater access to water (Fig. 3b). This is likely a consequence of trees with less access to ground water corresponding to warmer sites (Fig. 3c). Such trees will be more water-limited during the dry, Mediterranean summers that limit carbon gain (Hollinger 1992). Consequently, they will be more resource limited, and may leaf out earlier in order to use more surface soil water left over from the wet winter in order to compensate for lower carbon gain in the summer. Gene transcriptional responses to water stress in valley oaks affect over half its genome, indicating considerable opportunity for local adaptation that is likely to be the proximate driver of this relationship (Gugger et al. 2016).

Soil available phosphorus also correlated significantly with mean budburst date of individual trees. Trees growing in soils with more P, and thus enhanced availability of soil nutrients, leafed out earlier than trees with less access to P (Fig. 3b). This is also likely to be a consequence of microclimatic differences among sites. Soil microbial activity that causes litter decomposition is temperature dependent and warmer sites have faster rates of litter decomposition (Meentemeyer 1978). Although we do not have litter decomposition data, our results are consistent with previous work demonstrating that phosphorus is largely released from decomposing litter, whereas nitrogen is not (Knops et al. 2010). Thus, warmer sites with faster litter decomposition should have higher P, but not necessarily higher N. Mean budburst date of individual trees also correlated with elevation, with trees at higher elevation growing in sites with warmer winter microclimate and leafing out earlier. Tree size (dbh) did not correlate with budburst; previous work has shown considerable variability among species in terms of the effect of tree size on phenology (Marchand et al. 2020).

We found strong effects of budburst phenology on subsequent growth and reproduction of trees (Table 3). Confirming the convex-shaped trend found in earlier analyses (Koenig et al. 2012), trees leafing out relatively early and relatively late compared to the population mean had significantly reduced acorn crops the following autumn (Fig. 4a), a relationship consistent with pollen limitation playing an impor-

tant role in acorn production (Pesendorfer et al. 2016). More surprising was that radial growth was also greater among trees leafing out at or near the population mean budburst date (Fig. 4b). Thus, trees leafing out early in the season apparently did not benefit by acquiring more resources allowing them to either grow more or produce more acorns, as might be predicted if such trees are able to photosynthesize for a greater length of time. Countering this line of reasoning, previous work found that trees in this population leafing out earlier experienced greater rates of folivore damage than trees setting leaves later (Pearse et al. 2015b). In addition, early budburst entails a greater risk of frost harming flowers and subsequent seed production (Fowells and Schubert 1956). Clearly there are multiple factors affecting the relationship between phenology and fitness. In any case, we found strong selection against trees leafing out early (or late) within a year as evidenced by their relatively poorer radial growth and smaller subsequent investment in reproduction. The explanation for the greater radial growth in trees leafing out around the mean population budburst date remains to be determined.

In an analysis quantifying the relative importance of between-tree effects and within-tree plasticity, both significantly contributed to differences in acorn production (Table 3). Trees that leafed out at or near the population mean produced larger acorn crops than trees leafing out relatively early or late (between-tree effect). Among years, however, individual trees produced larger acorn crops when they leafed out at or near the population mean for the year compared to when they did not (within-tree effect). Only the latter within-tree effect, indicative of individual plasticity, contributed significantly to the effect of budburst timing on radial growth. These results support analyses using common garden plantings that have found considerable environmental plasticity in phenotypic variation in both valley oak (Wright et al. 2021) and the largely sympatric blue oak (Papper and Ackley 2021). Budburst phenology is yet another character of valley oaks, along with acorn size (Koenig et al. 2009), sex allocation (Knops and Koenig 2012), and a range of other life-history traits (Barringer et al. 2013), that exhibits wide variability both across years and among individuals.

Valley oaks have been aptly described as "...the monarch of California oaks by virtue of [their] size, age, and beauty" (Pavlik et al. 1991). Unfortunately, this species has suffered inordinately over the past 150 years due to agricultural and residential development and stands to lose an estimated 54% of its modern potential range as a consequence of climate change by the end of the 21st century (Kueppers et al. 2005). Phenology is thought to play a key role, not only as an indicator of climate change, but as a major determinant of plant species ranges (Chuine and Beaubien 2001). It remains to be seen whether the considerable variability in valley oak phenology

documented here will help this species adapt to the changing conditions of California’s future.

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TIMING OF BUD BURST IS ASSOCIATED WITH CLIMATE OF MATERNAL ORIGIN IN *QUERCUS LOBATA* PROGENY IN A COMMON GARDEN

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ABSTRACT

Deciduous trees leaf out in the spring beginning with bud burst. Proximally, the timing of that process is triggered by temperature, but natural selection on bud burst timing may have acted in local populations through additional factors, such as frost damage, insect herbivores and fungal pathogens. Valley Oak, *Quercus lobata* Née, is a deciduous California endemic tree species that shapes the ecosystems where it is found. We examined the phenology of spring bud burst in trees collected from 674 maternal families across the species range, grown in two replicate common gardens. We found significant differences among the families for timing of bud burst, and also found that onset of bud burst differed between gardens, presumably due to climate. The differences among families were associated with the climate of origin where the trees were collected suggesting that some extent of genetic differentiation in bud burst is due to local adaptation. Given predicted changes in climate in California for the future, understanding patterns of bud burst will help inform the selection of seed sources for reforestation efforts.

Key Words: climate change, genetic variation, leaf set, leaf unfolding, maternal variation, phenology, quantitative genetics, Valley Oak.

Phenology, the timing of events in a tree's annual life cycle, is critical for the fitness of the tree. Resulting from complex interactions between the environment and genetically controlled responses, phenology determines the length of the growing season and possibly the annual growth as well (e.g., Polgar and Primack 2011). Selection by many factors, such as frost damage, herbivory and foliar pathogens, can shape the timing of bud burst. Newly emerged leaves are generally highly sensitive to frost, such that selection associated with cold temperatures has shaped the timing of bud burst in several tree species (Vitasse et al. 2013; Augspurger 2009). In addition, several studies have reported damage by herbivores when leaves first emerge, indicating a relationship between phenology and levels of herbivore damage (Visser et al. 2006; Pearse et al. 2015; Wood and Pidgeon 2015). Finally, foliar pathogens, such as powdery mildew, have also been implicated in causing selection on the timing of leafing out in oaks (Dantec et al. 2015). In some cases, selection acts in opposition. For example, early emergence may create more risk of frost but may also result in less powdery mildew (Dantec et al. 2015). Additionally, selection to increase the number of days for leaves to photosynthesize through earlier bud burst

may be countered by selection to avoid early frost. Thus, the combination of these factors will affect growth in plants, which is an important component of fitness.

The extent to which geographical patterns of bud burst timing are genetically or environmentally influenced is key to discussions of assisted migration (Aitken and Bemmels 2016). When seeds are moved and planted into a new location, they carry the genetic information that adapted them to their home environment. The timing of bud burst in their new habitat will have a profound impact on their fitness going forward, particularly because the timing of bud burst and flowering is linked developmentally in reproductive oak trees. Understanding how the climate where an acorn is collected impacts the timing of bud burst in a new planting location will help inform seed transfer guidelines. Moreover, in a changing climate, knowing the relationship between climate of origin and timing of bud burst will allow for more nuanced seed transfer guidelines that take into account not only the current climate at a planting location, but also population data that are modeled based on predicted future climates (e.g., see Browne et al. 2019).

Valley Oak, *Quercus lobata* Née, is an iconic oak species, endemic to California (Pavlik et al. 1995). It is mainly distributed around the California central valley and is found as far south as the Transverse Ranges, and north past Redding (Griffin and Critchfield 1972). A winter deciduous tree, Valley Oak begins to leaf out in the early spring, though the extent of marcescence (the retention of dead leaves) varies within the species and populations (Sork and Wright unpublished data; Karban 2007). It is considered a foundational species and dominates the habitats where it is found. In addition, oaks are an important cultural and nutritional resource for Native American peoples (Anderson 2007).

To assess genetic differences and phenotypic variation among Valley Oak maternal families collected from localities (provenances) for a variety of traits, we established a provenance test in 2014 (Delfino Mix et al. 2015), using 2-yr old seedlings planted into two contrasting field sites: the Institute of Forest Genetics (IFG), Placerville, CA and the Chico Seed Orchard (Chico), Chico, CA. Every year since planting, we have recorded spring leaf emergence as a measure of tree phenology through weekly surveys of tree buds. We have observed significant genetic differentiation and phenotypic plasticity in a range of leaf traits and progeny height (MacDonald 2017), as well as climate-associated relative growth rates (Browne et al. 2019). Here, we focus on differences in phenological patterns within the species by addressing the following questions: (1) Is there genetic variation for the timing of bud burst in a common garden study? And (2) Is the timing of bud burst associated with climate of origin? We then discuss the genetic and environmental basis for this timing in oak management and restoration projects.

METHODS

Establishment of the Test- Acorn Collection, Planting Sites

In 2012, acorns were collected from 674 maternal trees at 95 different sites across the species range. Over 11,000 acorns were germinated at the USDA-Forest Service, PSW Institute of Forest Genetics in Placerville, CA (IFG) (38.740, -120.738) (see Fig. S1 in Browne et al. 2019 for a map). Details of the collections, germination and early growth are given in Delfino Mix et al. (2015). Nearly 7000 seedlings were planted in the field during the winter of 2014/2015 at two replicate sites: IFG and the USDA Forest Service Chico Seed Orchard (Chico) in Chico, CA (39.708, -120.780). Trees were irrigated each summer at both field sites. Weeds were controlled with a combination of herbicide and mechanical control.

Collection of Bud Burst Data

At the beginning of each growing season (approximately February 1), trees were scored weekly for

their leaf development (see Appendix S1 for sample sizes at each site and year). During each survey, we assigned each tree a score ranging from 0 (no sign of bud burst) to 5 (leaves fully unfolded), following the bud burst stages identified by Derory et al. (2006), who correlated differential transcriptome expression patterns with bud burst stages in *Quercus petraea* (Matt.) Liebl.

Climate Data

For each of the maternal family collection locational coordinates (GPS data were taken when acorns were collected), we obtained estimated historical climate data from the Basin Characterization Model (Flint et al. 2013) using the 1951 to 1980 30-yr average data. We extracted data for the following climate variables: bioclim_01 through bioclim_19, AET (actual evapotranspiration) and CWD (climatic water deficit) (<https://worldclim.org/data/bioclim.html>; Flint et al. 2013). Analysis revealed strong correlations among temperature and precipitation variables. We selected a set of climate variables to analyze that had correlations < 0.80 among variables: bioclim_02 (mean diurnal range), bioclim_04 (temperature seasonality), bioclim_05 (maximum temperature of the warmest month), bioclim_06 (minimum temperature of the coldest month), bioclim_13 (precipitation of the wettest month), bioclim_14 (precipitation of the driest month), bioclim_15 (precipitation seasonality), and AET (actual evapotranspiration). Only two sets of climate variables correlated with each other highly: bioclim_04 and bioclim_05 ($r = 0.705$) and bioclim_13 and AET ($r = 0.775$) (Appendix S2).

Data Analysis

For each tree at each site and year, we calculated the date that the first sign of bud burst was observed. Often that was Stage 1, but if the tree was developing quickly, it could have been a higher stage. That date was scored as number of days from January 1 and was used as the bud burst response for subsequent analyses. Chico and IFG had two differences that we accounted for in our analysis. At Chico, the trees were planted in three different planting areas across the site. In addition, there were multiple observers each year recording data. At IFG, however, there was a single planting area, and one primary observer.

To assess whether the maternal families varied for date of first bud burst and to account for possible effects of observer and planting area in Chico, we used a mixed model GLM (PROC MIXED in SAS 9.4, SAS Institute, Cary, NC; Littell et al. 1996), with year considered a fixed effect, and observer, planting area and maternal families as random effects. We included observer as a nested effect within year, as the observers differed each year. From this analysis, we estimated the Best Linear Unbiased Predictors (BLUPs) for each maternal family and used those

TABLE 1. GENERALIZED MIXED MODELS FOR DATE OF FIRST BUD BURST AND THE FIXED EFFECTS OF YEAR AND SITE. The model included the random effect of maternal family, which is more completely analyzed in Table 2.

Effect	df _{num}	df _{den}	F value	P-value
Year	1	9547	20.69	<0.0001
Site	1	9746	1714.94	<0.0001
Year × Site	1	9547	614.89	<0.0001

values for subsequent analyses. At IFG, a similar mixed model was run, but it did not include planting area or observer, simply year and maternal family, because there was only one primary observer and one planting area.

For both analyses, the “covtest” option was selected, giving a test of the significance of the random effects using a Wald Score. In addition, we confirmed the Wald Score results using a chi-square test of the differences between Log-Likelihood scores for models with and without the random effect of maternal family. The P-value, derived from a chi-square distribution with a degree of freedom = 1, was divided by two to make it a two-tailed test (Littell et al. 1996).

To estimate associations between parental climate and timing of bud burst in the two field sites, a multiple regression model selection was used, with the LASSO selection procedure (Efron et al. 2004). We ran models separately for each site, using BLUPs obtained for maternal families in the mixed model described above as observations for the maternal families in the experiment. BLUPs controlled for variation among observers, planting areas and years, and therefore represent the constitutive bud burst patterns of the maternal families. We included the selected climate variables described above, as well as the latitude, longitude, and elevation of the parental trees. To facilitate comparison among variables, we calculated standardized estimates for the predictors using the “stb” option in the model statement. For raw maternal family means and BLUPs as well as the complete data set see Appendices S3, S4 and S5 stored in FigShare (<https://doi.org/10.6084/m9.figshare.14524338>).

To test for a significant difference between planting sites and years and their interaction, we used a simplified mixed effect model, with the fixed effects of site and year, and the random effect of maternal family. Because the other sources of variation (planting area and observer) were not balanced between the two sites, we did not include them in the analysis. This model provides a conservative estimate of the effects of site and year, because not all sources of variation are included in it. However, the effects that were included in the model accounted for a sufficiently large portion of the variation that including any additional effects in the model would not change the interpretation of the results.

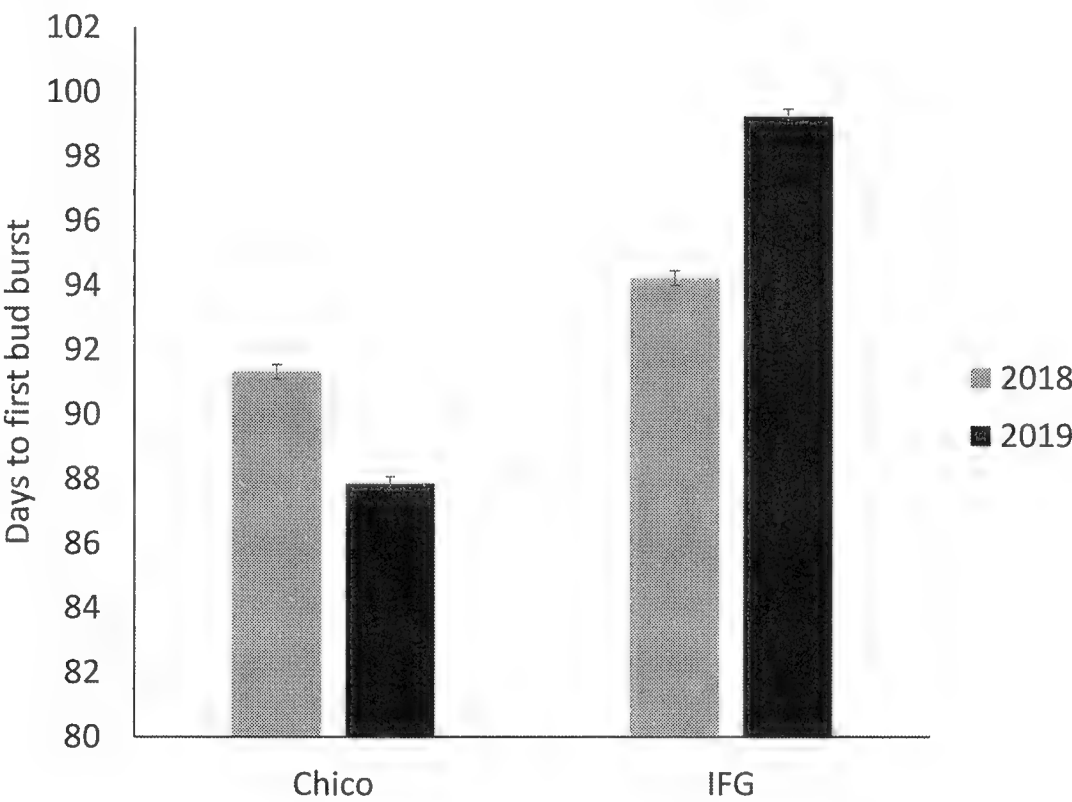


FIG. 1. Least squared means from a GLMM of the interaction between site and year from a model that included maternal family as a random effect.

RESULTS

The calendar day of initiation of bud burst ranged from 38–155 across the two sites and years (January 1 = day 1). At IFG, bud burst started later than Chico, with dates that ranged from 42 to 155, with an average of 94.1 (±0.14 SE) and 99.2 (±0.18 SE) in 2018 and 2019 respectively, while at Chico they ranged from 38–130 with an average of 91.3 (±0.21 SE) and 87.8 (±0.19 SE) in 2018 and 2019. We found significant variation in the timing of bud burst between sites and years, as well as an interaction between the two (Table 1, Fig. 1). Our comparison of variation in bud burst among the maternal families revealed a significant result for both the Wald Score test, as well as the Log-Likelihood Ratio Test at both sites, suggesting significant genetic variation among maternal families was observed for this trait (Table 2).

To assess whether differences among families were associated with climate of origin, we used multiple regression with the LASSO model selection technique, and we identified five climate variables, as well as elevation and longitude of the maternal trees that were associated with the date of first bud burst (Table 3, Fig. 2). Two of these were associated with precipitation (Bioclim_13 and 14 – precipitation of the wettest month and precipitation of the driest month, respectively), and two were associated with temperature (Bioclim_02 and 05 – diurnal range and maximum temperature in the warmest month). Evapotranspiration was also associated with bud burst, but only at the Chico site (Table 3, Fig. 2).

DISCUSSION

Our experiment demonstrates that variation in the timing of bud burst in Valley Oak is due to genetic differentiation. We found significant associations with climate, as well as the elevation and longitude where the trees came from, but not the latitude of

TABLE 2. GENERALIZED MIXED MODELS FOR DATE OF FIRST BUD BURST. A. CHICO. This model includes the fixed effect of year, and the random effects of maternal family, section and observer. B. IFG. This model includes the fixed effect of year and the random effect of maternal family. An asterisk (*) indicates results were confirmed using a Log-Likelihood Ratio Test (see Methods).

Site	Effect type	Effect	Estimate	Standard error	Z-value	P-value	Effect type	Effect	df _{num}	df _{den}	F value	P-value
A. Chico	Random	Maternal family	10.9282	1.2093	9.04	< 0.0001*	Fixed	Year	1	7.97	1.85	0.2107
		Section	5.8902	6.1423	0.96	0.1688						
		Observer (year)	14.5680	7.3774	1.97	0.0242						
		Residual	83.3455	1.7417	47.85	< 0.0001						
B. IFG	Random	Maternal family	19.7274	1.4632	13.48	<0.0001*	Fixed	Year	1	4299	698.23	<0.0001
		Residual	44.7832	0.9665	46.33	<0.0001						

their home origin, which indicates that local temperatures may have played a greater role in phenology than daylength. Differences among maternal families when grown in a common garden were statistically significant, which demonstrates a genetic basis to bud burst timing. A number of other studies have looked at the timing of bud burst in deciduous tree species grown in common gardens. Papper and Ackerly (2021, this issue) studying phenological variation in Blue Oak (*Quercus douglasii* Hook & Arn.) trees in a common garden found a strong, significant effect of both the planting environment and genetics. Alberto et al. (2011) documented significant heritability for bud burst in a European study of *Q. petraea* with 10 populations sampled across an altitudinal gradient, looking at 1-yr old seedlings growing in a greenhouse environment. In another study of seven tree species, Vitasse et al. (2013) observed strong evidence for genetic differences in bud burst, while Pearse et al. (2015) tested family effects in *Q. lobata* and also found evidence for genetic differences.

We found that the timing of bud burst was associated with climate, as well as the elevation and longitude of origin, which are likely to be surrogates

for climate variables. Alberto et al. (2011) also found that trees from warmer sites had earlier bud burst, as did Papper and Ackerly (2021, this issue). Higher elevation has been associated with later bud burst in a number of studies of trees grown in common gardens (Deans and Harvey 1995; Vitasse et al. 2009; Firmat et al. 2017). In addition, we found that trees from warmer environments had earlier bud burst,

TABLE 3. LASSO REGRESSION MODEL SELECTION RESULTS FOR EACH SITE. Chico adjusted $R^2 = 0.2778$; IFG adjusted $R^2 = 0.3408$.

Site	Parameter	df	Estimate
Chico	Intercept	1	0
	Longitude	1	0.119
	Elevation	1	0.275
	bioclim_02: Mean diurnal range	1	−0.150
	bioclim_05: Max temp warmest month	1	−0.121
	bioclim_13: Precip of wettest month	1	0.217
	Bioclim_14: Precip of driest month	1	0.056
	AET	1	0.042
IFG	Intercept	1	0
	Longitude	1	0.126
	Elevation	1	0.211
	bioclim_02: Mean diurnal range	1	−0.189
	bioclim_05: Max Temp warmest month	1	−0.223
	bioclim_13: Precip of wettest month	1	0.244
	Bioclim_14: Precip of driest month	1	0.066

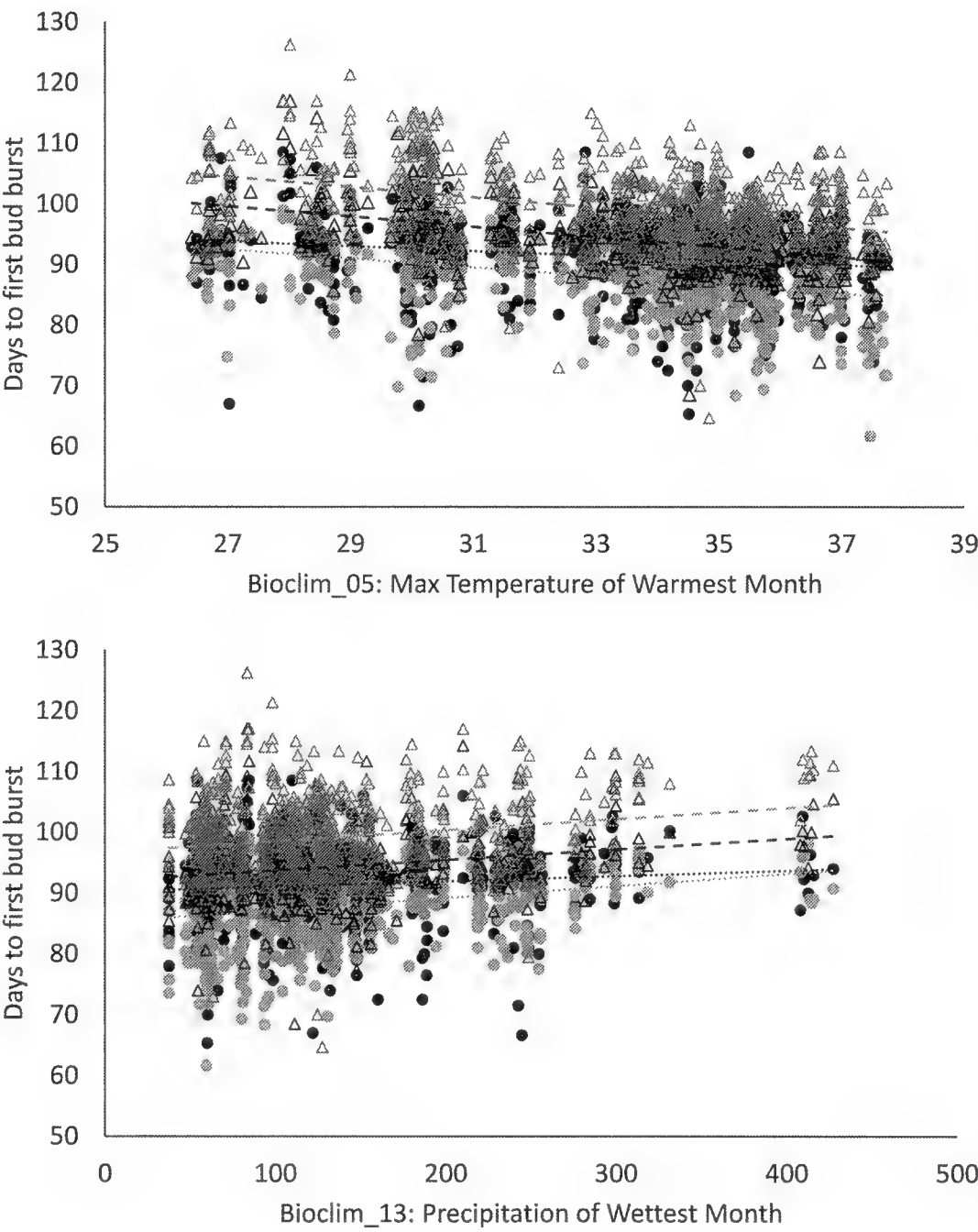


FIG. 2. Scatter plots of the average date of first bud burst for each maternal family of *Quercus lobata* grown in two replicate common gardens at each planting site and year for each source site variable (each marker represents the average of one family). A. Bioclim 5: Max Temperature of Warmest Month, B. Bioclim 13: Precipitation of Wettest Month. IFG is shown in open triangles, Chico is solid circles. 2018 is in black, and 2019 in grey. Linear regression lines: larger dashes show IFG, smaller dashes show Chico. 2018 is shown in black, and 2018 is shown in grey.

consistent with a number of other studies that have shown increased temperature of origin is associated with earlier bud burst (Alberto et al. 2011; Sampaio et al. 2016; Dewan et al. 2020).

Our data provide evidence that bud burst timing varies with the environment. We found that trees growing in Chico burst bud earlier in the year on average than trees growing at the higher-elevation, colder IFG site in both years. There is an approximate difference in elevation between the two sites of 770 m and 2.8 and 11.3 d between the average bud burst date for the two sites in 2018 and 2019 respectively. Vitesse et al. (2013) found similar results, with leafing out date later at higher elevation planting sites for seven trees species. Koenig et al. (2021, this issue) examined bud burst date in Valley Oak over a 30-yr period, and found that warmer temperatures were associated with earlier bud burst. Studies that used artificial warming to examine the impact of increased temperatures on phenology have found that warmer temperatures are associated with earlier leafing out dates (Morin et al. 2010; Fu et al. 2013; Fu et al. 2016; Dewan et al. 2020; Faticov et al. 2020). Thus, in addition to the genetic basis of bud burst timing, the environment can influence when leaves emerge.

Valley Oaks are threatened by climate change, wildfires and land use changes (Tyler et al. 2006; Sork et al. 2010). As such, they are often the focus of reforestation projects where acorns must be chosen for use in reforestation planting. Several factors can be considered in this choice. The first is climate of origin. Climate models predict increasing temperatures throughout California (Thorne et al. 2017), thus selecting trees adapted to a warmer climate may increase the success of reforestation projects. Given that such trees are likely to break bud earlier than locally-derived seedlings, they may benefit from a longer growing season than local genotypes. Although multiple studies have shown that earlier bud burst is associated with earlier fall leaf senescence in Eastern US and European deciduous trees (Keenan and Richardson 2015; Zani et al. 2020), our observations of Valley Oak saplings in the two common gardens indicated that overall bud break was earlier and marcescence was longer for all provenances at the warmer site (Wright and Sork unpublished data). Thus, the timing of bud burst seems to have both a genetic basis associated with maternal site and a degree of plasticity shaped by the temperature of the planting site. In terms of phenology, the biggest risk of planting progeny derived from warmer sites into cooler sites for the sake of future warmer climates would be the possibility of damage from a late frost in the spring, as well as being more vulnerable to early-season herbivory (Pearse et al. 2015). The extent of these problems would need to be assessed. A potential additional problem may be early flowering as the progeny become reproductive adults, given the fact that bud burst and flowering are developmentally

linked. Such early flowering may be asynchronous with the rest of the population and result in lower acorn production (see Koenig et al. 2012). Given the long flowering season of Valley Oaks (typically early February through early April; Lentz and Sork unpublished data) and the fact that restored populations may include individuals from many localities, the possible risk of asynchronous bud burst and flowering in human-managed populations may be less a concern relative to the benefits of genotypes adapted to warmer conditions. Nonetheless, projects considering assisted migration can keep these risks in mind. Phenology is clearly an important and complex trait to consider when selecting seeds for reforestation projects.

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THE ROLE OF WINTER PRECIPITATION AS A CLIMATIC DRIVER OF THE SPRING PHENOLOGY OF FIVE CALIFORNIA *QUERCUS* SPECIES (FAGACEAE)

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ABSTRACT

Understanding the phenological sensitivity of keystone plant species, such as oaks, to climate variables provides a foundation for assessing the impacts of changing climate on ecosystem resilience, biodiversity, and carbon sequestration. This study assessed the responsiveness of bud burst, flowering, and fruiting phenophases of five native California oaks to climate variables using 2012–2019 USA National Phenology Network data, which are contributed by scientists and trained volunteers. Climate data included seasonal measurements of precipitation and maximum and minimum temperatures. Phenophase data for five oak species: *Quercus agrifolia* Née and *Quercus kelloggii* Newb. from *Quercus* section *Lobatae* (red oaks), *Quercus douglasii* Hook. & Arn., *Quercus garryana* Douglas ex Hook., and *Quercus lobata* Née from *Quercus* section *Quercus* (white oaks) were analyzed. The majority of the trees in the study were located in California, although a small number of sites were beyond their normal distribution ranges. No significant differences were found among species for bud burst and fruiting onsets using ANOVA and Tukey HSD tests. However, significant differences were identified between the flowering onsets of the white oaks *Quercus lobata* and *Quercus douglasii* and between *Quercus lobata* and the red oak *Quercus agrifolia*. Multiple regression models identified the strongest climate predictors of oak phenophase onset as: (1) winter precipitation, (2) mean accumulated precipitation, and (3) maximum winter temperature, so that winter precipitation and temperature have been found to be the main climate drivers of vegetative growth and reproductive potential for these native California oaks.

Key Words: California oaks, climate drivers, community science, Nature's Notebook, phenology, phenophase onset, *Quercus*, USA National Phenology Network.

Plant phenology is the study of the onsets and durations of growth and reproductive cycles such as bud burst, flowering and seed dispersal. When measured over time, phenological data can provide important information regarding plant responses to changes in climate variables, such as temperature and precipitation (Schwartz 2003; Chuine 2010). Phenophase timing shifts are important indicators of climate change, particularly in California's water-limited Mediterranean climate (Lipton et al. 2018). This highlights the importance of monitoring the phenological sensitivity of keystone species, such as oaks, which support high levels of biodiversity, carbon sequestration, and ecosystem resilience (Bargali et al. 2015).

Species have evolved traits to respond to temperature cues and increasing daylength with the early or late onset of bud burst, balancing access to resources, such as light and nutrients, against the risk of frost damage (Chamberlain et al. 2019; Wright et al. 2021). Species with traits for chilling requirements during dormancy are generally better protected from late season frosts by halting bud burst until the chilling requirement has been met (Ford et al. 2016). Additionally, species with strong daylength requirements, which delay bud burst, are less likely to be impacted by frosts, but therefore have limited responses to take advantage of early warming (Chamberlain et al. 2019). Spatial differentiation of phenological responses also occurs within species. For example, populations at higher elevations or latitudes may initiate early onset upon meeting their

chilling requirements, while those at lower latitudes, whose chilling requirements may be unmet, exhibit delayed or failed bud-break, or the opposite may occur with lower elevation populations responding to warmer temperatures with earlier onsets than higher elevation populations (Ford et al. 2016). Similarly, the timing of flowering onset and pollen dispersal underlies reproductive success, requiring trade-offs between maximizing phenophase duration and minimizing risk factors to seed and fruit maturation (Koenig et al. 2012).

Oaks are adapted to maximize their use of resources and may exhibit polycyclism, or multiple flushes of leaves or flowers throughout the growing season, responding opportunistically to precipitation and temperature changes. California oaks in particular are adapted to taking advantage of limited and varying amounts of precipitation (Bobinac et al. 2012; Gerst et al. 2017). Onset dates of bud burst and the subsequent length of the growing season are important factors for tree species since they determine the potential amount of height and radial growth available for that year (Askeyev et al. 2005; Ford et al. 2016; Koenig et al. 2021). Multiple flushes may also be responses to herbivory or infectious agents, such as oak moth defoliation or powdery mildew which may also be influenced by climate variables themselves, stimulating trees to produce young leaves to compensate for the loss of large proportions of their leaf canopy (Visser and Holleman 2001).

TABLE 1. VARIABLES INCLUDED IN THE DATASETS FROM THE USA-NPN (2020) AND THEIR DEFINITIONS.

Variable	Definition
Latitude	latitude of the observation site
Longitude	longitude of the observation site
Elevation (m)	elevation of the observation site
Mean First Yes DOY (onset)	mean first observed positive phenophase onset at that site, day of the year 1–366
Mean Last Yes DOY	mean last observed positive phenophase observation at that site, day of the year 1–366
Onset year	year of observation
T _{max} Winter (°C)	average maximum winter temperature for the onset year (December of previous year to February of onset year)
T _{max} Spring (°C)	average maximum spring temperature for the onset year (March–May)
T _{max} Summer (°C)	average maximum summer temperature for the onset year (June–August)
T _{max} Autumn (°C)	average maximum temperature for the previous autumn before onset (September–November)
T _{min} Winter (°C)	average minimum winter temperature for the onset year (December of previous year to February of onset year)
T _{min} Spring (°C)	average minimum spring temperature for the onset year (March–May)
T _{min} Summer (°C)	average minimum summer temperature for the onset year (June–August)
T _{min} Autumn (°C)	average minimum temperature for the previous autumn before onset (September–November)
Prcp Winter (mm)	accumulated precipitation for the previous winter before onset (December of previous year to February of onset year)
Prcp Spring (mm)	accumulated spring precipitation for the onset year (March–May)
Prcp Summer (mm)	accumulated summer precipitation for the onset year (June–August)
Prcp Autumn (mm)	accumulated precipitation for the previous autumn before onset (September–November)
Mean Accum Prcp (mm)	mean accumulated precipitation from 1st of January to the onset day of the year
Mean Daylength	mean number of seconds of daylength for onset day of the year
Mean AGDD	accumulated growing degree days from January 1st – accumulated maximum temperature when (T _{max} + T _{min})/2 > 0°C for onset day of the year

Previous studies have identified temperature as a driver of oak phenology, with Askeyev et al. (2005) reporting that early spring warming resulted in earlier bud burst onsets in *Q. robur* L. in Russia, while the role of precipitation has been unclear. Furthermore, Gerst et al. (2017) compared two central and eastern North American oak species (*Q. alba* L. and *Q. rubra* L.) with two western species (*Q. agrifolia* Née and *Q. lobata* Née) using 2009–2014 USA-NPN citizen science data and identified seasonal temperature variables as being the most significant drivers of leaf and flower flushes for the western oaks, while noting that precipitation was a stronger driver in the eastern species’ models while the western species exhibited low sensitivity to precipitation.

There are 20 native Californian oak species, with additional hybrids, occupying overlapping distributions varying with abiotic factors including elevation, aspect and soils (Nixon 2002). High levels of morphological variation occur within and between *Quercus* species, with the majority of Californian species being from the white oak clade, *Quercus* section *Quercus*, or from the red oaks, *Quercus* section *Lobatae* (Hipp 2015).

This study aimed to expand our understanding of the phenological responsiveness of five of California’s dominant native oaks by using large community-contributed science datasets to assess whether there were significant differences among species’ phenophase onsets. Multi-variable models of the

drivers of those phenophases were then developed to identify the strongest explanatory climate variables.

METHODS

USA National Phenology Network Phenometric Data

Phenology data have been contributed to the California Phenology Project, as part of the USA National Phenology Network (USA-NPN), since 2009. The USA-NPN offers a database whereby professional and community scientists regularly record long-term phenological observations using standardized protocols for over 1200 floral and faunal taxa, including 26 oak species, via Nature’s Notebook (Rosemartin et al. 2018). The USA-NPN observation protocols are rigorous and provide data products of phenophase status and abundance of marked plants along with their latitude, longitude and elevation (Rosemartin et al. 2018).

Additionally, the USA-NPN offers climate variables originating from Daymet, as gridded daily surface weather and climate data, to provide spatially explicit data, including measures of daylength and calculations of aggregated values, such as Accumulated Growing Degree Days (AGDD) and seasonal average temperature and precipitation measurements (Thornton et al. 2016; USA-NPN 2020). Table 1 summarizes the geospatial and climate variables used in this study. Additionally, statewide California

TABLE 2. MEAN ANNUAL TEMPERATURE AND PRECIPITATION FOR CALIFORNIA 2010–2018 AND COMPARISONS WITH 1901–2000 MEAN VALUES, WHICH WERE CALCULATED USING THE STATEWIDE TIME SERIES TOOL (NOAA 2019).

Year	Mean annual temperature (°C)	Difference from 1901–2000 mean of 14.1°C	Mean annual precipitation (cm)	Difference from 1901–2000 mean of 56.87cm
2010	14.28	+0.18	79.20	+22.33
2011	14.11	+0.01	47.73	−9.14
2012	15.28	+1.18	59.56	+2.69
2013	15.17	+1.07	20.14	−36.73
2014	16.33	+2.23	50.55	−6.32
2015	16.00	+1.9	38.20	−18.67
2016	15.61	+1.51	65.35	+8.48
2017	15.72	+1.62	71.55	+14.68
2018	15.61	+1.51	45.67	−11.20
2010–2018 mean	15.35		53.11	
Mean difference from 1901–2000 baseline		+1.25°C		−3.76 cm

mean annual temperature and precipitation data for 1902 to 2018 were accessed using NOAA’s statewide time series tool (NOAA 2019) to compare recent measures with historical trends (Table 2).

Firstly, the five oak species with the highest numbers of observations within California (2011–2019) were identified in the USA-NPN database: *Q. agrifolia* Née., *Q. douglasii* Hook. & Arn., *Q. garryana* Douglas ex Hook., *Q. lobata* Née., and *Q. kelloggii* Newb. Four of the species were deciduous, with the single evergreen species being *Q. agrifolia*. The majority of the trees in the study were located in California, although a small number of sites were beyond their normal distribution ranges, including some *Q. garryana* and *Q. kelloggii* in Oregon and eight *Q. agrifolia* in Massachusetts and North Dakota. The onsets of bud burst, flowering (of the male inflorescences, or catkins), and fruiting were selected as the focus of this study, based on the day of the year when a new phenophase was observed for each plant, following clearly defined phenophase descriptions (USA-NPN 2020). Bud burst was observed when a distinct leaf tip emerged from a leaf bud. Flowering was observed with the emergence of flower buds or the presence of inflorescences, and fruiting was noted when juvenile acorns were seen to have developed (USA-NPN 2021).

The five oak species datasets were downloaded from the USA-NPN’s database and imported into R-Studio (R version 3.5.1). Observations were selected with an accuracy of seven days, whereby only ‘Yes’ observations preceded by ‘No’ observations within seven days were analyzed; this is the highest level of accuracy offered in identifying phenophase onset dates and reduces the incidence of data entry errors or of onsets missed because of infrequent observations. Partial years of data were discarded and, in order to focus on spring phenophase onsets, each bud burst and flowering dataset was filtered to include only days 1–180 of each year (DOY 1–180) between 2012–2019. For fruiting, days 1–30 of each

year were filtered out of datasets to exclude observations before flowering had occurred and which were therefore likely to be non-viable fruit retained from the previous season which had become visible with leaf drop.

One of the species, *Q. kelloggii*, was problematic regarding fruiting data since these acorns require an 18-month maturation period, rather than the more common one year. Consequently, *Q. kelloggii* may bear two generations of acorns simultaneously, making it difficult to identify whether observations are for new juvenile acorns or whether onsets of new fruits have been masked by the ongoing presence of the previous season’s fruits. *Q. kelloggii*’s fruiting dataset was also very small. Consequently, all fruiting phenophases of ‘fruit’ and ‘ripe fruit’ were combined and included in *Q. kelloggii*’s analysis.

Data Analysis

Firstly, one-way ANOVA tests (followed by Tukey HSD Tests when appropriate) were used to compare the phenophase onset means of the five species between 2012 and spring 2019 to assess whether there were significant differences among the species’ means. Within R-Studio *Tidyverse* (version 1.2.1) packages were used for data analysis and for linear modeling of the data (Wickham and Grole-mund 2017).

Secondly, multiple linear regression models were developed using the bud burst, flowering, and fruiting datasets’ climate (such as average seasonal maximum (T_{max}) and minimum temperatures (T_{min}) and accumulated seasonal precipitation) and geo-graphical variables (such as latitude, longitude, and elevation) to assess which were the strongest predic-tors of phenophase onsets. The explanatory climate and geographical variables were scaled, by centering around the means, to ensure that the estimated regression coefficients used the same scale (between 0 and 1) and to allow comparison of effects. Nested multiple linear regression models were created,

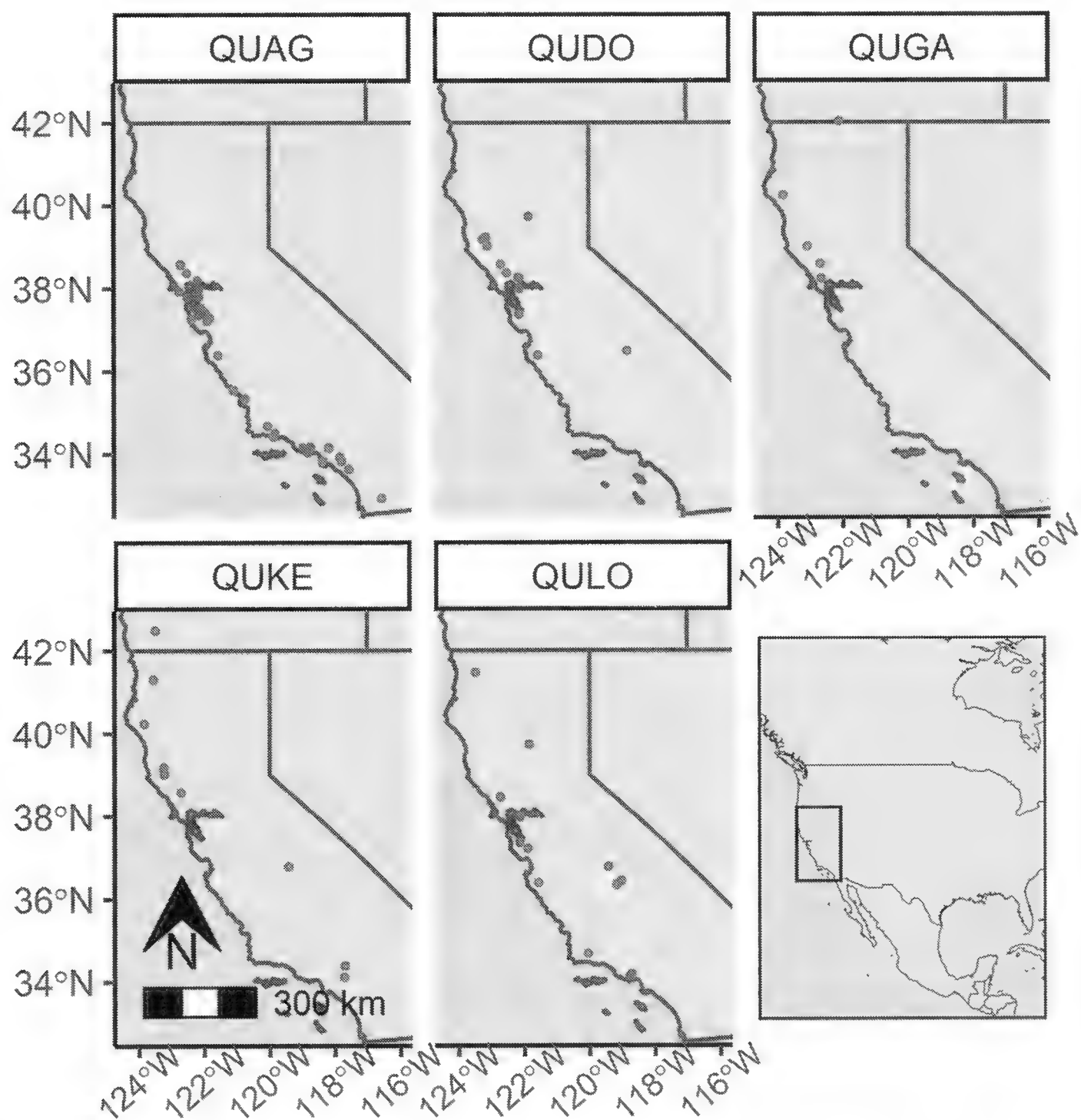


FIG. 1. Maps showing locations of study observation sites for each oak species in California, with *Q. garryana* and *Q. kelloggii* including sites in Oregon (USA-NPN 2020). Note that eight *Q. agrifolia* observations were also from two sites in Jamestown, N. Dakota and Boston, Massachusetts not shown on the maps. QUAG: *Q. agrifolia*, QUDO: *Q. douglasii*, QUGA: *Q. garryana*, QUKE: *Q. kelloggii*, QULO: *Q. lobata*. Maps created with Natural Earth.

whereby the response variable was phenophase onset and the explanatory variables included all climate and geographical variables, aiming to assess which variables had the greatest influence on the fit of the models to the phenology data by sequentially removing variables and comparing models.

Models were compared by choosing the model with the lowest Akaike's Information Criterion (AIC) value, then removing the explanatory variable with the highest P-value to produce the next model. Residual plots were checked to assess the fit of the models and ANOVA was used to check homogeneity of variance and to compare succeeding models. Model residuals were plotted using normal quantile-quantile plots to identify deviations from homoscedasticity. Confidence intervals were calculated and variance inflation factors (VIF) were used to assess multicollinearity between variables, with VIF values above five indicating collinearity. Models with the lowest AIC and variables having significant P-values ($\alpha = 0.05$) were selected to produce the best-fitting models. Coefficient plots were used to illustrate the explanatory power of the climate variables in the best-fitting models with the strongest variables being further away from the zero line. Narrow confidence intervals represent higher degrees of certainty about the strength of the models' predictive power for the variables.

RESULTS

Fig. 1 shows the locations of each species' observation sites. Most study sites were based throughout California, however some extended northwards since the species distribution ranges of both *Q. garryana* and *Q. kelloggii* occur in southern Oregon. Additionally, two sites with eight *Q. agrifolia* observations in Boston, MA and Jamestown, ND were included in these analyses (USA-NPN 2020). Table 3 shows the geographical ranges of latitude, longitude and elevation for each of the five species with the overall range being 32.9° to 46.9° for latitude, -124.3° to 71.1° for longitude, and 0 to 2053m for elevation.

The earliest mean onsets for all oak species were seen for all phenophases during the peak of the 2012–2016 drought, whereby the earliest bud burst and flowering onsets occurred in the spring of 2015 following a rainy winter and the highest average temperatures during the study period. In 2015, California's average temperature was 1.9°C above the 1901–2000 mean, precipitation was 18.67 cm below the baseline, and the mean bud burst onset of all combined species occurred on day of the year 52, followed by flowering onset on day 62, and fruiting onset on day 138 (Table 2, NOAA 2019).

TABLE 3. RANGES OF LATITUDE, LONGITUDE, AND ELEVATION FOR USA-NPN OBSERVATION DATA.

Species	Latitude range	Longitude range	Elevation range (m)
<i>Q. agrifolia</i>	32.9°–46.9°	–122.7° to –71.1°	0–1255
<i>Q. douglasii</i>	36.4°–40.7°	–123.2° to –118.8°	17–536
<i>Q. garryana</i>	38.2°–42.5°	–124.3° to –122.1°	39–958
<i>Q. kelloggii</i>	33.8°–42.5°	–123.8° to –116.7°	61–2053
<i>Q. lobata</i>	34.1°–41.5°	–123.5° to –118.7°	45–632

The mean phenophase onsets (day of the year) between 2012–2019 for the five combined species were 71.302 (SE = 1.344, n = 382) for bud burst, 77.033 (SE = 1.502, n = 322) for flowering, and 156.544 (SE = 3.582, n = 297). Median phenophase onsets for each species were illustrated using box plots (Fig. 2).

No significant differences were identified among bud burst onset means for individual species ($F_{4,378} = 1.058$, $P = 0.377$,) (Table 4). No significant difference was identified among the mean onsets of the five species for fruiting ($F_{4,293} = 1.942$, $P = 0.104$). However, significant differences were identified for mean spring flowering onset among the five species ($F_{4,318} = 4.906$, $P < 0.001$). *Quercus lobata* had an earlier onset of flowering than *Q. agrifolia* (Tukey’s HSD Test, $P = 0.026$) and *Q. lobata*’s onset was earlier than *Q. douglasii*’s (Tukey’s HSD test, $P = 0.001$).

First model outputs for each phenophase identified the primary role of geographic factors, rather than climate variables, in predicting phenophase onset; daylength, latitude, elevation, and longitude had the greatest predictive weights in determining phenophase onsets and producing high R^2 values. Since this study was focused on climate drivers of phenophases, the geographic variables were removed from subsequent nested models leaving only the climate variables, such as T_{max} , T_{min} and seasonal cumulative precipitation values.

Table 5 illustrates the outputs of the best-fitting linear models for the onsets of each phenophase. The strongest explanatory climate variables (Fig. 3) were found to be winter precipitation, mean accumulated precipitation, and winter T_{max} .

The best-fitting bud burst model with eight climate predictors (winter precipitation, mean accumulated precipitation, winter T_{max} , summer T_{max} , summer T_{min} , spring precipitation, autumn T_{min} , autumn precipitation) explained 37% of the variance (Table 5). The strongest flowering model included seven predictors (winter precipitation, mean accumulated precipitation, winter T_{max} , autumn T_{max} , summer T_{max} , autumn precipitation, summer T_{min}) and explained 28% of the variation in phenophase onset. The best fruiting model was less predictive despite including eight explanatory variables (winter precipitation, mean accumulated precipitation, winter T_{max} , autumn precipitation, autumn T_{min} , spring T_{max} , spring precipitation, summer T_{min}), explaining only 11% of the effect.

Coefficient plots for bud burst and flowering models demonstrate that the temperature and precipitation variables generally have narrow confidence intervals without crossing the zero line (Figs. 3A and 3B). However, in contrast, the fruiting model has wider confidence intervals and three variable coefficients cross the zero line, illustrating the weaker predictive power of the model (Fig. 3C).

Overall, winter precipitation, mean accumulated precipitation and winter T_{max} were identified as the most significant climate drivers of bud burst, flowering and fruiting phenophases for these five California native oaks.

DISCUSSION

The earliest mean onsets for all five oak species were seen for all phenophases during the peak of the 2012–2016 drought, whereby the earliest bud burst and flowering onsets occurred in the spring of 2015 following a rainy winter and the highest average temperatures during the study period. In 2015, California’s average temperature was 1.9°C above the 1901–2000 mean, precipitation was 18.67 cm below the baseline, and the mean bud burst onset of all combined species occurred on day of the year 52, followed by flowering onset on day 62, and fruiting onset on day 138 (Table 2, NOAA 2019). Koenig et al. (2021) similarly reported that *Q. lobata* exhibited earliest bud burst onset in this same year, 2015, in a 30-year study of this species in central coastal California.

California oaks are adapted to taking advantage of limited and varying amounts of precipitation, as has been demonstrated in this study with the identification of winter precipitation as the most significant climatic driver of spring bud burst, flowering and fruiting. No significant difference was found among the five oak species’ bud burst or fruiting onsets (Table 4, Figs. 3A and 3C), suggesting that they are generally adapted to respond to the same climate cues. This effect was seen across both oak clades, the white oaks, *Quercus* section *Quercus*, and the red oaks, *Quercus* section *Lobatae*, including four deciduous species and the single evergreen species, *Q. agrifolia*.

Significant differences were, however, found for flowering onset between the species, particularly between *Q. lobata* and *Q. agrifolia* and between *Q. lobata* and *Q. douglasii*. *Quercus kelloggii*’s median flowering onset was earlier than that of the other oak species, followed by *Q. agrifolia*, *Q. lobata*, *Q.*

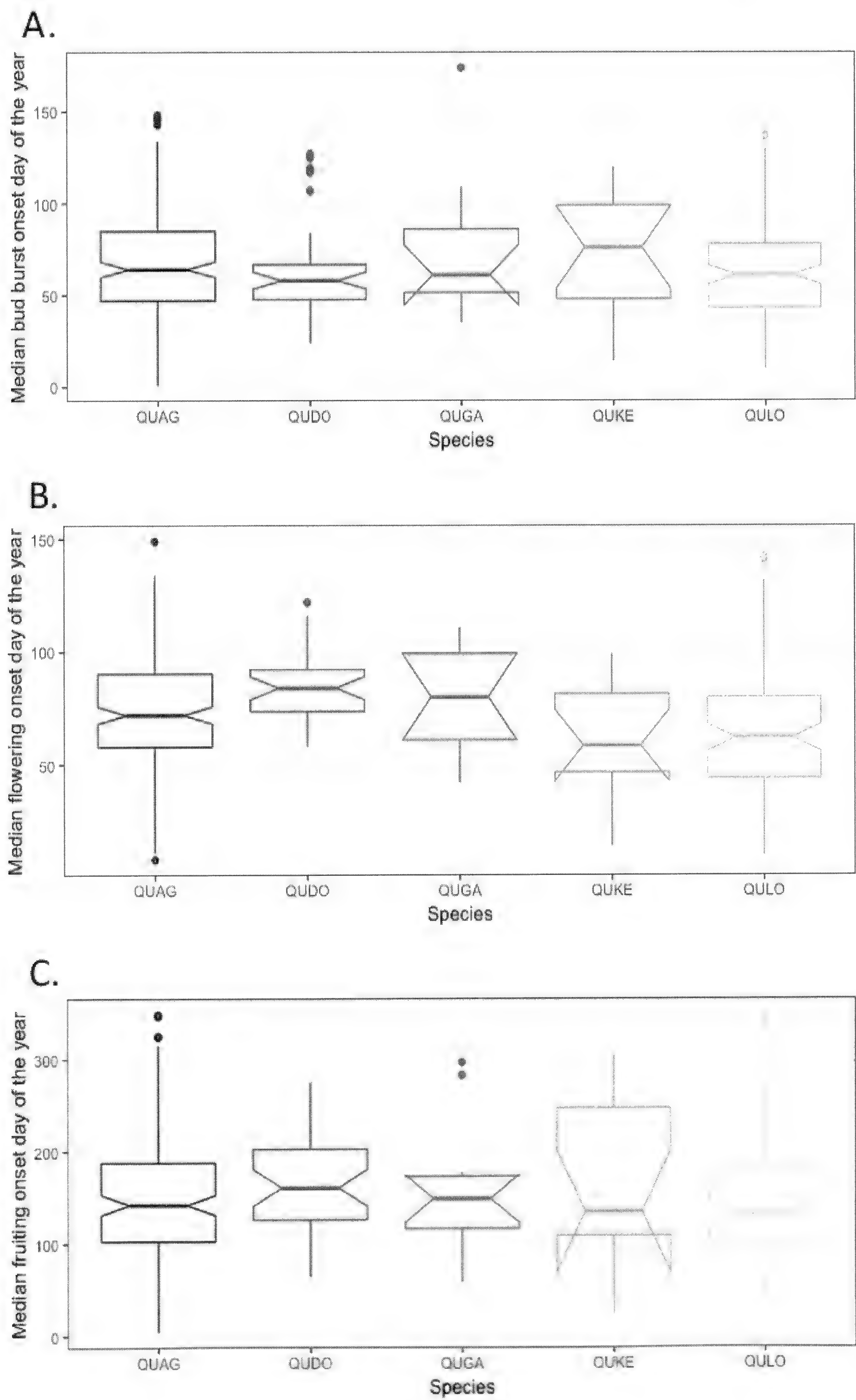


FIG. 2. Box plots of median phenophase onset for five native California oak species, 2012–2019, showing the minimum, first quartile, median, third quartile and maximum for each set of onsets. A. Bud burst. B. Flowering. C. Fruiting. *QUAG*: *Q. agrifolia*, *QUDO*: *Q. douglasii*, *QUGA*: *Q. garryana*, *QUKE*: *Q. kelloggii*, *QULO*: *Q. lobata*.

TABLE 4. ANOVA AND TUKEY HSD RESULTS TESTING FOR SIGNIFICANT DIFFERENCES ($\alpha = 0.05$) AMONG MEAN PHENOPHASE ONSETS OF FIVE NATIVE CALIFORNIA OAK SPECIES USING USA-NPN DATASETS BETWEEN 2012–2019.

Phenophase	Species (sample size)	df	F-ratio	P value	Adjusted P value between species if < 0.05
Bud burst onset	<i>Q. agrifolia</i> (205)	4	1.058	0.377	
	<i>Q. douglasii</i> (42)				
	<i>Q. garryana</i> (11)				
	<i>Q. kelloggii</i> (13)				
	<i>Q. lobata</i> (111)				
	Total = 382				
Flowering onset	<i>Q. agrifolia</i> (180)	4	4.906	< 0.001	QULO-QUDO: 0.001 QULO-QUAG: 0.026
	<i>Q. douglasii</i> (32)				
	<i>Q. garryana</i> (10)				
	<i>Q. kelloggii</i> (12)				
	<i>Q. lobata</i> (88)				
	Total = 322				
Fruiting onset	<i>Q. agrifolia</i> (156)	4	1.942	0.104	
	<i>Q. douglasii</i> (37)				
	<i>Q. garryana</i> (12)				
	<i>Q. kelloggii</i> (11)				
	<i>Q. lobata</i> (81)				
	Total = 297				

garryana and then *Q. douglasii* (Table 4, Fig. 2B). The relative timing of bud burst and flowering varies, with some oak species tending to produce new leaves shortly before staminate flower buds become apparent, while in others leaves and catkins emerge synchronously since terminal buds are frequently mixed, containing both new leaves and male flowers (Oliveira et al. 1994; Keator 1998). The discrepancy between the timing of bud burst and flowering noted here may be attributed to the effect of non-flowering trees on the dataset, since populations may include many non-flowering trees, which may not yet have reached maturity or resulting from other unknown factors, and subsequently will not go on to produce any fruits (Koenig et al. 2012).

Temporal differentiation of flowering and pollen-release periods between species lessens the likelihood of hybridization in mixed species communities, since large amounts of pollen are not being dispersed simultaneously (Cavender-Bares et al. 2017). Hybridization does not occur between *Quercus* clades, but *Q. lobata* and *Q. douglasii* are both in the white oak clade, so that hybridization may occur between these species when their distributions overlap (Hipp et al. 2018). The significant difference between the flowering onsets of these two white oak species may be a genetic adaptation maintaining differentiation between them and reducing the probability of cross pollination and hybridization. Cavender-Bares et al.

(2017) attributed temporal segregation in recruitment and regeneration between the lineages as an enhancement towards coexistence between the clades, by maintaining speciation through reproductive isolation, illustrated here by the differing flowering onsets of the white oak *Q. lobata* and the red oak *Q. agrifolia*.

However, *Q. garryana* is also in the white oak clade and has an overlapping flowering phenophase with both white oak species, increasing the probability of hybridization between them. This may explain the occurrence of many hybrid populations of white oaks, including Epling’s oaks, *Quercus* × *eplingii*, which are hybrids between *Q. douglasii* and *Q. garryana* and commonly occur in communities throughout California, such as in blue oak woodland alliances (CNPS 2019).

Model outputs for each phenophase identified the primary role of geographic factors rather than climatic variables in predicting phenology; day-length, latitude, elevation, and longitude had the greatest predictive weights in determining phenophase onsets and producing high R^2 values. This concurs with a study by Gerst et al. (2017) which compared the phenophases of western (*Q. lobata* and *Q. agrifolia*) and eastern US oaks (*Q. robur* and *Q. alba*) to assess the effects of species relatedness with geospatial factors and found that species were more

TABLE 5. OUTPUTS FOR THE BEST-FITTING PHENOPHASE ONSET LINEAR MODELS FOR FIVE CALIFORNIA OAK SPECIES USING USA-NPN DATA, 2012–2019.

Phenophase model	Coefficient estimate	Standard error	t-value	Residual error	Adjusted R^2	F-statistic	AIC	P value
Bud burst onset	71.302	1.344	53.043	23.05	0.3681	$F_{8,317} = 24.67$	2982	< 2.2e-16
Flowering onset	77.033	1.502	51.304	22.95	0.2801	$F_{7,267} = 14.84$	2514	< 2.2e-16
Fruiting onset	156.544	3.582	43.704	60.65	0.1061	$F_{8,273} = 6.185$	3119	2.41e-07

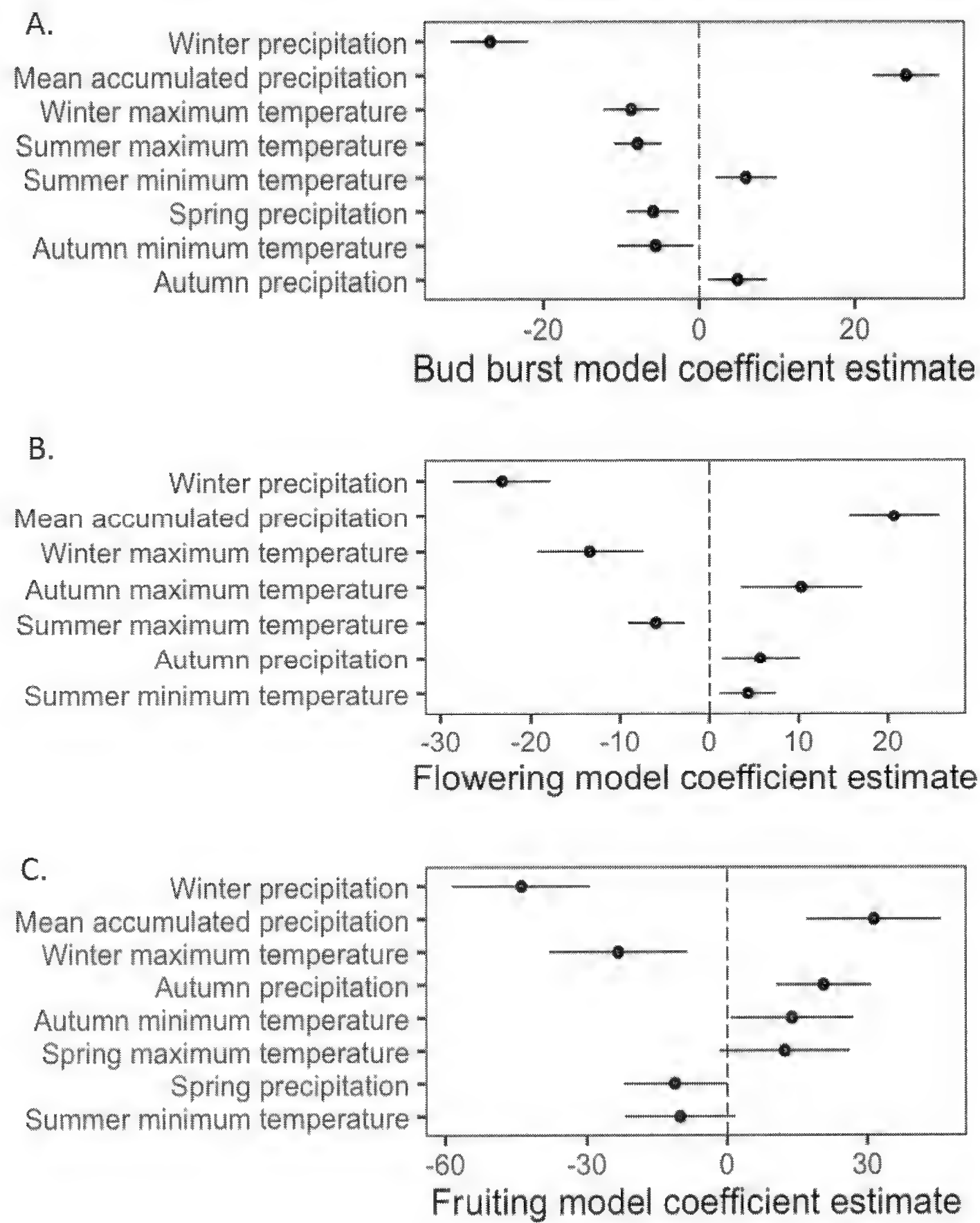


FIG. 3. Coefficient plots for model parameter estimates for climate drivers of California oak phenophases, showing coefficient estimates and their upper (97.5%) and lower (2.5%) confidence intervals. A. Bud burst. B. Flowering. C. Fruiting.

responsive to geographic factors than to relatedness between species.

Multiple regression modeling, after removing the geographical variables, identified winter precipitation and mean accumulated precipitation as the most significant climate variables for the onset of all three phenophases, with winter T_{max} being the most predictive temperature variable. Winters are often the most unpredictable season of the year in California, with swings in precipitation varying from drought conditions to seemingly constant rain or snow. Even without the additional uncertainties of climate change, it is not unexpected that this phenological sensitivity was exhibited by all five species across all phenophases (Swain et al. 2018).

The roles of maximum winter and spring temperatures have been previously identified as strong climatic drivers of the timing of oak phenophases although the role of precipitation has been less clear. For example, Askeyev et al. (2005) reported that winter temperatures in the Volga region of Russia had shifted bud burst 2.8 days earlier for every 1°C of warming for oaks and noted that temperatures in the

month before bud burst were the most significant predictors of this phenophase. Recently, Koenig et al. (2021) confirmed that warmer temperatures induced earlier bud burst in *Q. lobata*, while noting the inverse relationship between higher mean maximum temperatures and rainfall in California. Papper and Ackerly (2021) similarly identified warmer November and March minimum temperatures as drivers of advancing bud burst onsets.

Gerst et al. (2017) demonstrated that traits such as bud, flower, and fruit phenophases are extremely sensitive to temperature, or growing degree days, and that higher temperatures may induce early bud burst and leaf flushing in western tree species such as *Q. lobata* and *Q. agrifolia*, while eastern oaks such as *Q. robur* and *Q. alba* were more responsive to spatial and temporal precipitation variations. However, a comparison between the 2017 study and this one highlights significant differences between them. For example, Gerst et al. (2017) used a smaller dataset for *Q. lobata* and *Q. agrifolia* which included California data between July 2011–2014, with 2012 and 2013 being the coolest years during 2012–2018 and

California's 2012 average precipitation was 104.7% of average, while 2013 and 2014 were drought years with approximately 35% and 89% respectively of average precipitation (Table 2, NOAA 2019). Thus, the longer dataset used in this current project (2012–2019) includes the years before, during, and after the 2012–2016 drought with three additional rainy winters. This offers a longer dataset and a more representative sample of California oaks' responses to climate extremes, particularly regarding precipitation, which was identified as the dominant climatic driver of their phenophases in this study.

General limitations of plant phenology studies to predict the relationships between climate and the timing of phenophases, stem from the complex effects of many genetic, environmental and ecological variables, which can confound assumed relationships between climate and phenophases. These include community interactions, herbivory, disease, pests, pollinators, soil, nutrient and water availability, and anthropogenic impacts such as habitat fragmentation and pollution may also be significant (Knapp et al. 2001; Schwartz 2003). High estimates of variance, or R^2 values, were therefore not expected with these models owing to these complex ecosystem dynamics also influencing phenophase timing.

Strengths of this study include the relatively large datasets (1001 observations) in comparison with many phenological studies, stemming from the observations of community-contributed scientists and the widespread distribution of observation and climate data across Californian sites (in addition to southern Oregon, North Dakota, and Massachusetts) with the inclusion of five native oak species.

Concerns about the USA-NPN datasets and their usage in this study stem from the relatively short duration of the monitoring period. The larger *Q. agrifolia* and *Q. lobata* datasets began in 2012, but data collection began in 2014 for the other species, reducing observation numbers, particularly for *Q. kelloggii* and *Q. garryana*. Sample size was also an issue in this study, despite the large dataset offered by USA-NPN. Many of the total observations downloaded from the USA-NPN were of phenophases not being assessed here, such as pollen release and leaf senescence, so that some species had only one or two observations of individual phenophases in a single year. Inequities in the weighting of species further complicated analysis of variations in phenophase onset between species, for example, *Q. agrifolia* comprised 156 of the 297 total fruiting observations, while *Q. garryana* contributed only 12. Larger datasets with more frequent observations would greatly improve certainty about the differences between phenophases and responsiveness to climate cues. Adapting the initial data analysis parameters by loosening the requirement for only accepting 'Yes' observations which had been preceded by a 'No' observation within seven days (for example by increasing it to 14 days) would have increased the number of phenophase onset datapoints and thus

sample sizes, but may also have increased the inaccuracy of those onset dates.

Additionally, there are some concerns about the accuracy of the data. Many of the observation sites are located at University of California field stations or at scientific or educational organizations, so that many observers are scientists, researchers and educational staff, but many are community-based scientists with varying levels of experience (USA-NPN 2020). Although they are all trained to follow clear protocols, it is difficult to ensure that everyone is following them exactly. Good eyesight combined with careful use of binoculars or hand lenses is essential for the differentiation of whether a leaf bud is truly breaking or whether a catkin flower is open and producing pollen. Male flower buds are also easy to miss during the first days of bud break without experience, particularly when they emerge from mixed terminal buds along with young leaves which may obscure them (Keator 1998). This may lead to the onset of flowering being missed and reported as occurring after leaf bud burst. Lastly, the identification of new fruits is difficult because they are small and are initially difficult to differentiate from axillary buds, especially when high up in a tree. However, the USA-NPN strives to ensure standardization and accuracy by providing training and support; reporting 91% concordance between trained and expert observers and the flagging of conflicting observations (Fucillo et al. 2014).

The frequency of observations, or lack thereof, may also impact whether the onset or duration of a phenophase has been captured, especially for flowering which may be short and easily missed. These limitations may impact the accuracy of the data, particularly for those species, such as *Q. kelloggii* ($n = 36$, as compared with $n = 541$ for *Q. agrifolia*) with smaller numbers of observations which are indicative of fewer, less experienced observers. Weekend bias has also been postulated in community-based observations, where volunteers may be more likely to contribute to projects on weekends, thus increasing the likelihood of biased onset dates being noted, although Courter et al. (2013) reported that changes in habits seemed to be reducing this tendency.

A final note on possible inaccuracies in observational data concerns hybridization, which is a common feature of oak communities throughout California, particularly where white oak species distributions overlap. Oak hybrids abound while leaf morphology varies highly across individual trees and throughout populations, increasing the difficulty of differentiating between species and hybrids (Hipp 2015). Consequently, it may be assumed that a number of the trees identified by species in the USA-NPN database are actually hybrids, perhaps leading to a greater probability of similar phenophase onsets and durations. This may be a subject for further study.

Further study of the impacts of climate change and extreme weather events on phenophases is

critical to understanding the potential impacts on species regeneration and distributions as well as on forest resilience and dynamics, particularly with climate models projecting increasing frequency of droughts and extreme weather events like atmospheric rivers (Chuine 2010). The future of keystone species such as oaks is dependent on their adaptability to changing climate, and to climate extremes in particular. This adaptability rests on their phenological responsiveness, and winter precipitation has been found here to be a main driver of vegetative growth and reproductive potential. Those populations that are able to respond to changing climate variables by bringing forward or delaying phenophases, such as bud burst, flowering or fruit ripening, will be the most resilient and able to expand or maintain their ranges, while those less adaptable species will be unable to survive increasingly rapid rates of change.

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ABSENCE OF FLOWERING SHIFTS IN *ARCTOSTAPHYLOS* AND *CEANOOTHUS* OVER THE PAST CENTURY OF CLIMATE WARMING

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ABSTRACT

Flowering phenology in five chaparral species was investigated using more than a century of data obtained from herbarium collections. Three species examined were from the genus *Arctostaphylos* (Ericaceae) and two from *Ceanothus* (Rhamnaceae). Collections of these species were examined relative to climate change data during the same time period. For all the species, no change in average flowering time occurred during the past century. Considerable variability was found in flowering phenology and this variability was explored using generalized linear (GLM) and generalized linear mixed models (GLMM) and different dimensions of temperature and precipitation timing. While the genera performed differently, both required combinations of precipitation, temperature, and their interactions to predict flowering date. *Arctostaphylos* responded the most to precipitation interactions, while *Ceanothus* responded the most to temperature interactions and the previous growing season's precipitation. In both genera, regression coefficients were combinations of both positive and negative variables, indicating that flowering dates are complex interactions among the different dimensions of precipitation and temperature.

Key words: chaparral, climate change, Ericaceae, generalized linear mixed models, phenology, precipitation, temperature, Rhamnaceae.

Plants adjust to seasonal changes in their environments by modifying the timing of developmental stages (Forrest and Miller-Rushing 2010; Piao et al. 2019; Dorji et al. 2020). Developmental processes like bud-break, flowering, and maturing of fruit track changes in their environments (Menzel and Fabrian 1999; Walther et al. 2002; Parmesan and Yohe 2003; Root et al. 2003; Badeck et al. 2004; Menzel et al. 2006). The environmental processes influencing their phenology are multiple, but in humid, temperate regions, temperature is usually designated a principal regulatory process, often as a chilling requirement or as a constraint, along with photoperiod as a control on temperature fluctuations at the atypical time period (Körner and Basler 2010). In semi-arid or arid regions, precipitation, with its contribution to soil moisture, becomes a significant and sometimes a principal influence on phenology, as long as other aspects of the environment are not a constraint (Beatley 1974; Kemp 1983; Bowers and Dimmett 1994; Gordo and Sanz 2005; Lesica and Kittelson 2010; Cleland et al. 2012; Mazer et al. 2015). For example, summer flowering in desert 'sky islands' was a response to the amount and timing of monsoonal rains (Crimmins et al. 2011).

Most studies have indicated an advance in flowering phenology, or earlier flowering, in reaction to current changes in climate due to warmer winters and springs (Menzel et al. 2006; Gordo and Sanz 2010; Cleland et al. 2012; Mazer et al. 2013). Others have found more complex responses, such as delays in spring phenology (Yu et al. 2010) or divergence of responses in plants of the same community (Sherry et al. 2007; Cleland et al. 2012; Kopp et al. 2020). Early spring flowering plants have responded more sensi-

tively to warming and advanced their flowering compared to later blooming taxa (Park et al. 2019). Plants appear to be responding to multiple environmental influences, integrating those influences in sometimes unexpected responses (Peñuelas et al. 2004; Crimmins et al. 2010; Gordo and Sanz 2010).

California is experiencing increasing temperatures, while precipitation has fluctuated considerably, but has stayed roughly constant on average (Hayhoe et al. 2004; Dettinger 2005, 2016; Cayan et al. 2008) (Fig. 1). California already experiences extremely variable storm regimes (Ralph and Dettinger 2012), yet meteorological models indicate that this interannual-interdecadal variability will increase in the near future (Dettinger and Cayan 2014; Dettinger 2016; Gershunov et al. 2019). While climate change is quite apparent at higher latitudes, in western North America it has also become increasingly variable (Dettinger and Cayan 2014; Gershunov et al. 2019). If precipitation and temperature are important influences for plant phenology, phenological responses should respond to this environmental variability.

The objective of this study is to focus on a few woody species found in chaparral in the genera *Arctostaphylos* (Ericaceae) and *Ceanothus* (Rhamnaceae) and to investigate influences on their flowering phenology. Both genera are early bloomers and fall into a phenological class that is usually most sensitive to warming (e.g., Park et al. 2019). But flowering phenology is under the influence of a number of processes and in our Mediterranean climate, the amount and seasonality of water should influence flowering as much as temperature, especially in woody plants (Rathcke and Lacey 1985; Law et al. 2000; Grossiord et al. 2017). The first objective is to

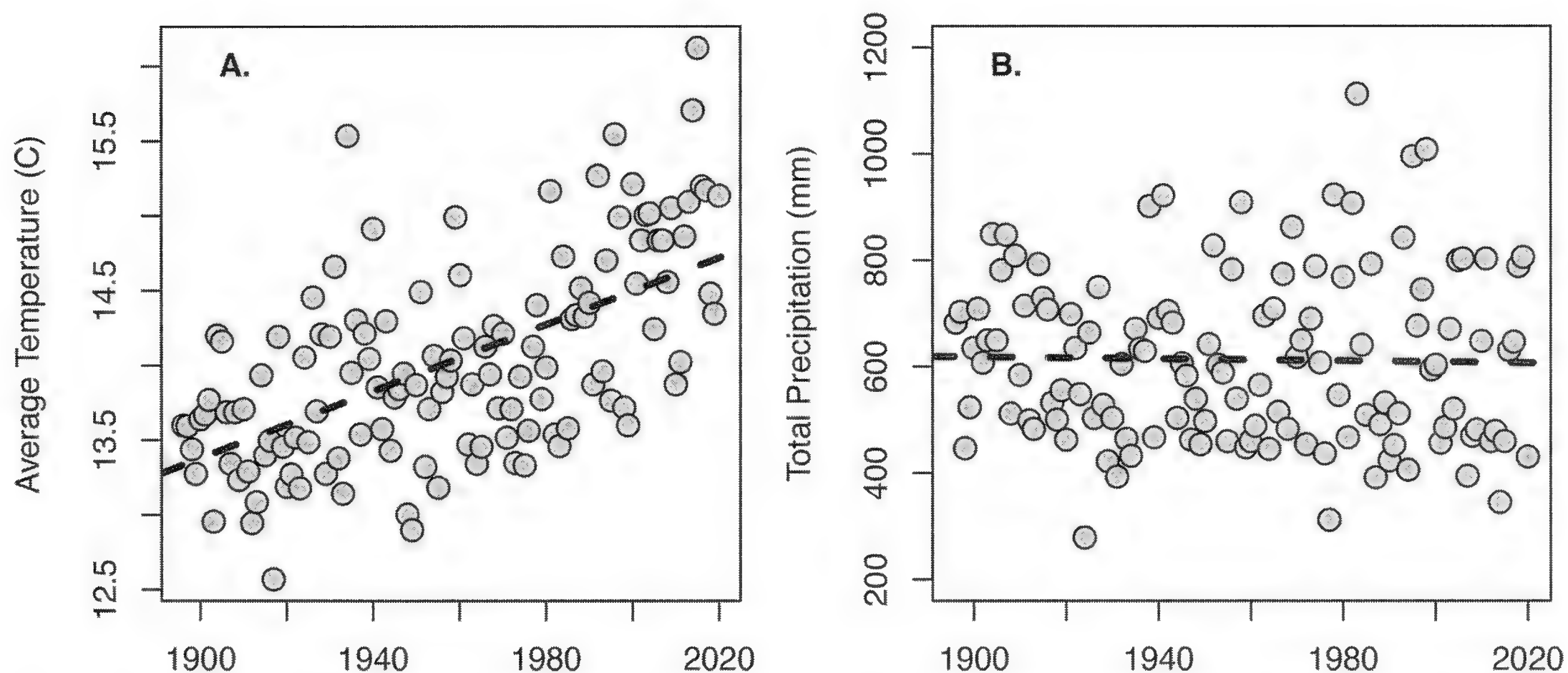


FIG. 1. Variation and trends in temperature and precipitation in California. A) Average temperature from 1886–2019; B) Total precipitation (mm) from 1886–2019. These data represent averages for each year based on data from the Western Regional Climate Center's (U.S.) WestWide Drought Tracker (<https://wrcc.dri.edu/wwdt>) that uses PRISM data (Abatzoglou et al. 2017).

assess whether species have responded to the past century's increase in winter/spring warming by advancing their flowering. A second objective is to determine the extent to which either temperature or precipitation during the growing season just before flowering is important, and whether there is an interaction between temperature and precipitation. Finally, in relatively seasonally dry habitats like California chaparral, one little explored issue is whether the prior year's precipitation might also have an influence. For example, a wet year should allow plants to produce and store more carbohydrates, potentially permitting earlier or more extensive flowering the following year, while a significant drought might limit resources in the next year and delay flowering.

METHODS

Species Selection

Arctostaphylos and *Ceanothus* were chosen as study taxa because of their overall distribution and dominance in the California Floristic Province, and because they differ in their typical flowering dates; *Arctostaphylos* species tend to flower between December and February, while *Ceanothus* species flower from March into April. Species of interest in this study included three species of *Arctostaphylos* (*A. glandulosa* Eastw. subsp. *glandulosa*, *A. andersonii* A.Gray, *A. hookeri* G.Don) and two species of *Ceanothus* (*C. cuneatus* (Hook.) Nutt., *C. thyrsiflorus* Eschsch.). These taxa were chosen for several reasons, one was to examine broad-ranging taxa (*A. glandulosa*, *C. cuneatus*), along with more narrowly restricted coastal taxa (*A. andersonii*, *A. hookeri*, *C. thyrsiflorus*). Herbarium collections were used as the source of date of

flowering, although a constraint was finding sufficient numbers of collections spread over a large number of years for plants in flower. A previous study determined that different lineages of *Arctostaphylos* species differed in phenology and the tetraploids also differed from one of those lineages (Parker et al. 2020). Therefore, species selected for this study represent those two lineages, plus the third is a tetraploid. The two *Ceanothus* species also represent the two large clades of this genus. Herbarium specimens have been found to be accurate with sufficient numbers for phenological work (Robbirt et al. 2011; Willis et al. 2017; Panchen et al. 2019).

Flowering Based on Collection Data

Flowering was the only phenological stage examined in this study spanning the years 1896–2020 using herbarium collections. If flowers were present, for each species the collection date and location data were recorded. In addition, collections were restricted to 0–1000 m elevation to ensure relatively similar phenological responses. Herbarium sheets were examined in person (California Academy of Sciences, CAS/DS); or digitally online (UC/JEPS, DAV, RSA/POM, and a few others using the Consortium of California Herbaria 2 database). Using these criteria, a large number of collections were analyzed ($n = 311$), specifically, for *C. thyrsiflorus* ($n = 102$); *C. cuneatus* ($n = 71$); *A. andersonii* ($n = 28$); *A. hookeri* ($n = 34$); *A. glandulosa* subsp. *glandulosa* ($n = 76$). Collections used are listed in Appendix 1.

Data Organization

The herbarium collection date was used as the sample date for flowering and converted to Day of Year (DOY) numbering. Because many *Arctostaphylos*

los species initiate flowering in the late fall-early winter and to restrict a single flowering season to be in a simple numerical sequence, collections dates were converted to a modified Day of Year (DOY) numbering scheme where October 1 corresponded to Day 1, and September 31 of the following year corresponded to Day 365 or 366, (equivalent to the ‘Water Year’ for the United States [USGS 2016]). Individual collections were excluded with dates earlier than 1896 due to the limitations of the environmental data set. The modified DOY scheme was also used for *Ceanothus* for consistency in statistical analyses.

Environmental Climate Data

Climate data were obtained from WestWide Drought Tracker (Abatzoglou et al. 2017), which uses data from the PRISM data set as a product of the Western Regional Climate Center (U.S.), the Desert Research Institute, and the University of Idaho. For the two broadly distributed taxa (*A. glandulosa* and *C. cuneatus*), latitude and longitude were extracted from the herbarium sheets; these were used to extract localized environmental data for each collection (nearest 4×4 km area). For the narrowly distributed taxa (*A. andersonii*, *A. hookeri*, *C. thyrsiflorus*), county averages encompassing their distribution range were used.

Previous studies indicate that precipitation and temperature for the growing season and immediately prior to flowering can influence flowering timing (Schlesinger et al. 1982; Keeley, 1987a). One study suggested that flowering in *Ceanothus*, at least, may be influenced by the previous growing-season’s total precipitation (Keeley and Keeley 1988). Based on these earlier studies, several temperature and precipitation measures were used to study their relative influence on flowering. These represent a preliminary investigation and are not intended as to suggest a final resolution of abiotic influences on flowering in these two genera.

Rather than using calendar years that cut across two growing seasons, the water-year (October 1–September 31) was used as the growing season year. Based on the water-year, for each individual collection, five climatic data values were obtained. Two temperature values used in analyses represented deviations from “normal” average temperature as provided by WestWide Drought Tracker, which used a 20-yr record to determine the normal averages (1981–2000; Abatzoglou et al. 2017). Deviations indicate cooler or warmer periods based on the averages for the location of each individual collection and permit a single comparative value across collection sites. The first temperature variable obtained for each collection was the deviation from average annual temperature based on a 12-mo water-year period. A second temperature value extracted was the deviation for just the months prior to flowering (a 5-mo period, October–February, for *Arctostaphylos*; a 6-mo period, October–March, for *Ceanothus*).

Three precipitation values were extracted as well; these values represent precipitation as a % of normal average precipitation; paralleling the temperature values, the ‘normal’ average was based on a 20-yr record (1981–2000), and data extracted for this study were based on the water-year sequence of months. The first precipitation value extracted was the 12-mo water-year % of normal encompassing the flowering date. The second value was the % normal precipitation for the water-year months prior to flowering (October–February for *Arctostaphylos*; October–March for *Ceanothus*). In addition to these two values, a third value representing the % normal precipitation for the previous water-year was also obtained for each collection site and date.

Statistical Analyses

Flowering dates were initially probed using simple linear regression analysis against environmental variables using the *lm* function in R (version 3.5.3, R Foundation for Statistical Computing, Vienna, Austria). To address the long-term data set as a group for each genus, generalized linear mixed models (GLMM) tested the date of flowering against the individual climate variables or in combinations with and without interactions, resulting in 40 different models tested. In these models, the fixed variables were single or combinations of the five environmental variables extracted from WWD/PRISM, and different species were considered random variables within each genus. The two genera were analyzed separately. The models used the *lme* function in R with from the *nlme* package. The Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to rank models. AIC and BIC estimate the relative quality of statistical models for a single data set; the lowest possible AIC or BIC indicates the best balance of model fit with generalizability. Residuals for the best model for each genus were graphed to examine whether the mean of the errors approximated zero, the distribution was normal, and the variation of errors appeared constant. The residuals were then specifically tested for normality using the Jarque-Bera Test (*jarqueberaTest* function) from the *fBasics* package. To analyze each species individually, generalized linear models were used (*glm* function in R), and were applied to the same 40 different models of individual climate variables or combinations; AIC was used to rank models in these analyses.

RESULTS

Temperature and Precipitation Trends

Temperatures have increased significantly on average over the last century in California (Abatzoglou et al. 2017) (Fig. 1a) (multiple $R^2 = 0.358$, adjusted $R^2 = 0.353$, $F_{1,123} = 68.62$, $P = 1.704e-13$), and the amount of variation among years has stayed

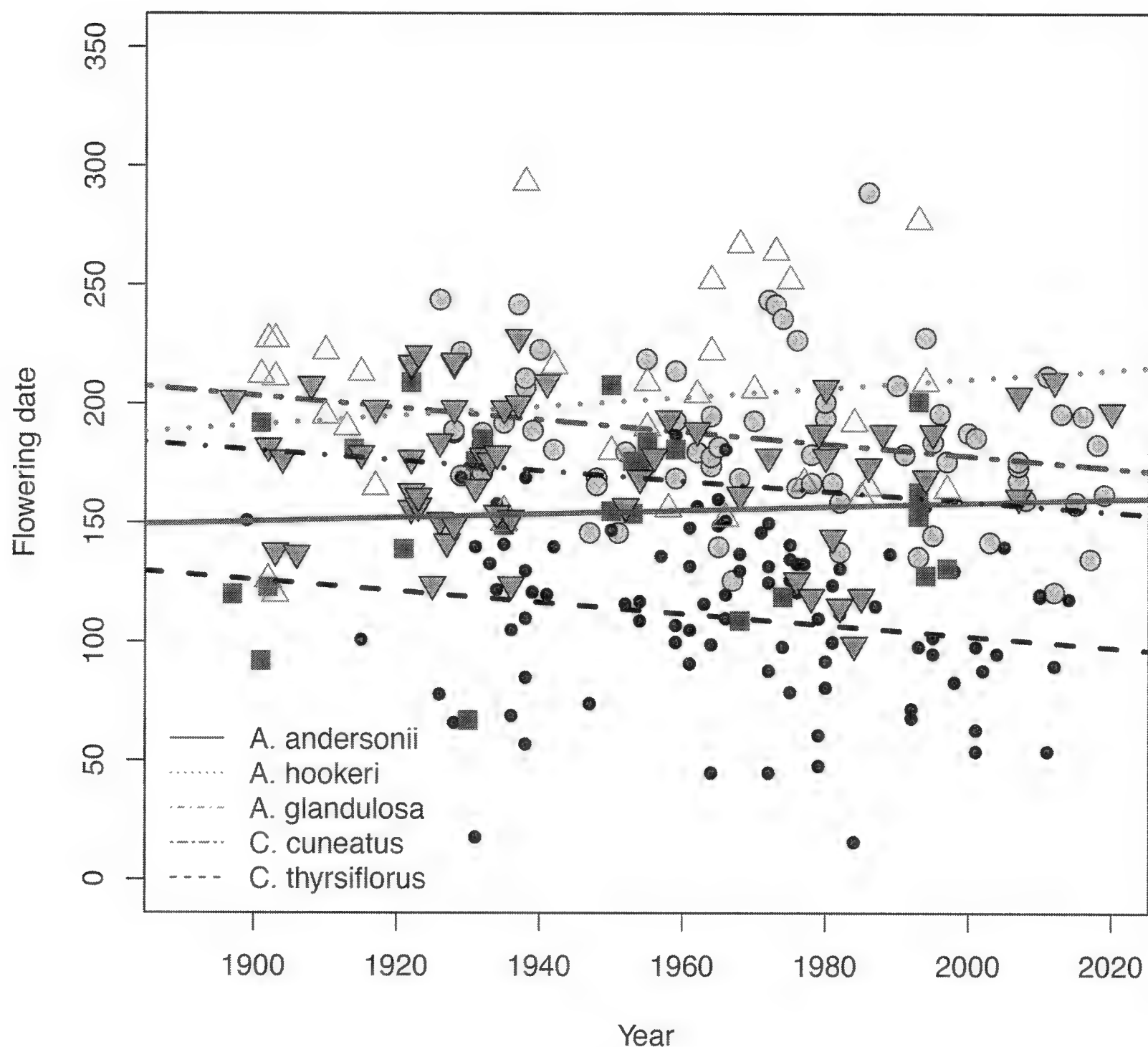


FIG. 2. Linear regressions of collection date for flowering individuals against time. No regression was statistically significant. Species symbols: *Arctostaphylos andersonii* are filled squares; *A. hookeri* are open triangles; *A. glandulosa* are downward and filled triangles; *Ceanothus cuneatus* are filled circles; *C. thyrsiflorus* are small, filled circles. Flowering date represents actual Day of Year for the two *Ceanothus* species. For *Arctostaphylos* species, due to flowering some years as early as November or December, a modified Day of Year (DOY+122) was used for this figure.

roughly constant. In contrast, precipitation has fluctuated considerably, but the average has changed little over the past century (Fig. 1b) (multiple $R^2 = 0.00043$, adjusted $R^2 = -0.0077$, $F_{1,123} = 0.053$. $P = 0.818$); although there is a trend for increased variability among years.

Linear Regressions

For all of these species, no trend in flowering phenology across the past century existed (Fig. 2). As could be predicted from the spread of data, r^2 results for these linear regressions were extremely low and ranged below 0.1. Linear regressions revealed that no species had significant relationships between flowering phenology and year, temperature departure from normal, % of normal precipitation, growing season temperature departure from normal, or growing season % of normal precipitation, and no linear relationship with the previous growing season's precipitation (Figs. 2–4).

Generalized Linear Mixed Models

In the GLMM models, simple single variable models for each genus performed poorly and rarely yielded statistically significant relationships between

flowering phenology and the variables. The best performing models were combinations of fixed variables and often the more complex ones that included interactions among variables (Table 1) (Burnham et al. 2011). Graphing the residuals for the best model suggested the mean of the errors approximated zero, the distribution was normal, and the variation of errors appeared constant. Therefore model assumptions were met. The Jarque-Bera Test for normal distribution of the residuals confirmed the best model for each species contained normal distribution; the distribution is normal to approximately normal if the P-values are not significant x (*Arctostaphylos*: $\chi^2 = 3.4829$, $P = 0.1753$; *Ceanothus*: $\chi^2 = 0.5177$, $P = 0.7719$).

Examining the top ranked models for each genus (Table 1) indicated that both genera are responding to temperature and precipitation, but their responses differ. For *Arctostaphylos*, all models emphasized different dimensions of precipitation as the most influential variables; generally, models combined interactions of the previous year's total precipitation with current season's precipitation. One highly-ranked model included current season temperature as influential. Coefficients for the best models were examined to understand the pattern associated with these environmental factors (Table 2). Positive

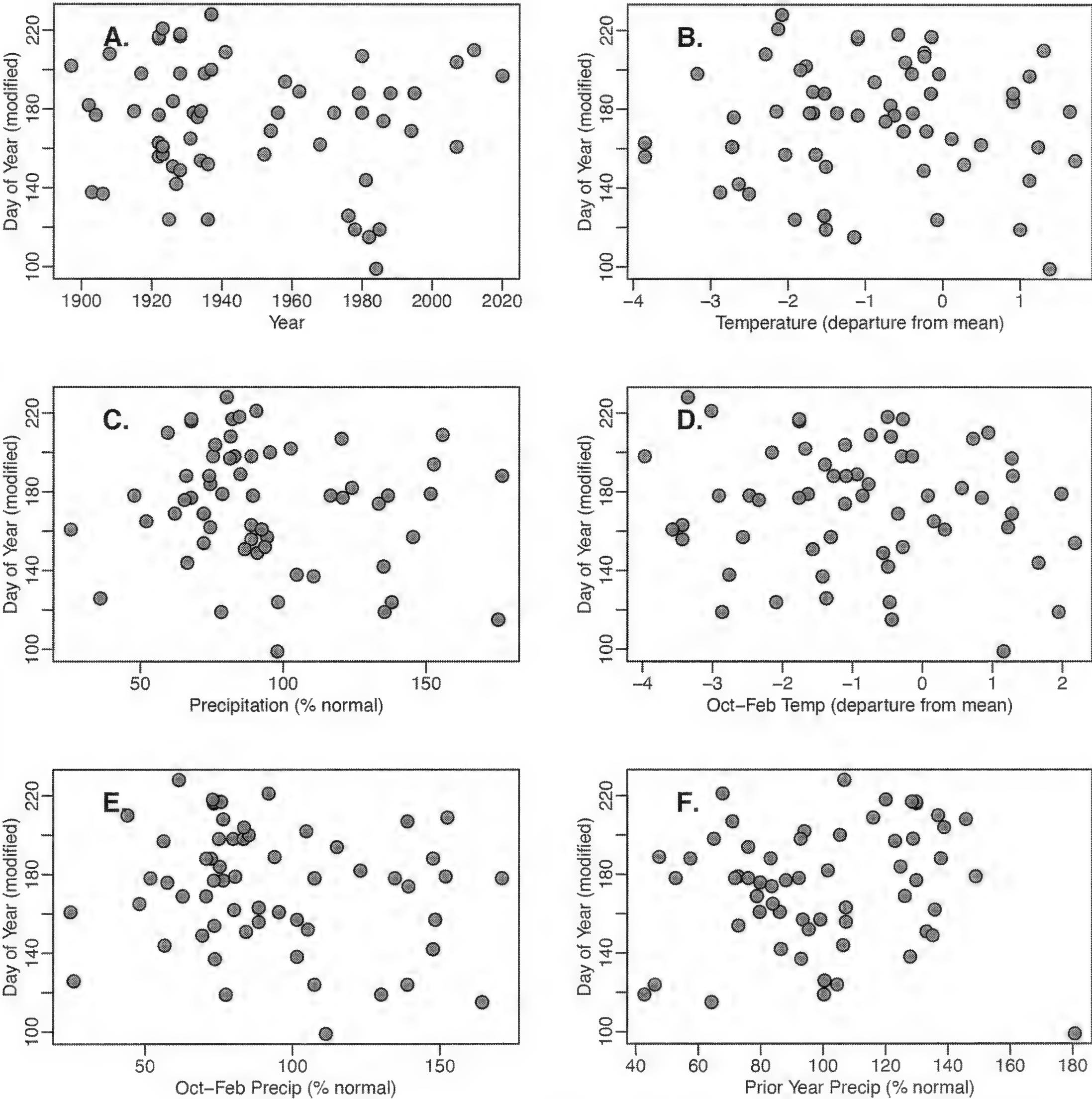


FIG. 3. Day of year flowering for *Arctostaphylos glandulosa* based on herbarium collections and various climatic factors. A modified flowering Day of Year (actual DOY+122) versus A) year of collection; B) departure from the mean average temperature for a given year; C) percentage of normal precipitation for a 12-mo period; D) departure from the mean average temperature for a 5-mo period before mean flowering; E) percentage of normal precipitation for a 5-mo period before mean flowering; F) percentage of normal precipitation for a 12-mo period prior to the year of collection.

coefficients will delay flowering while negative coefficients indicate earlier flowering. Because these are models with multiple variables, often a balance occurs between coefficients of the different climate variables supporting earlier and delayed flowering with the larger coefficients having greater impacts than smaller coefficients. Current season precipitation in the few months before flowering was usually the largest coefficient influencing flowering date. In the best model for *Arctostaphylos*, current season and prior season total precipitation were the most important influences and were positive, indicating

delayed flowering. Total season precipitation and interactions between current growing season precipitation and that of the previous year were negative and would advance flowering earlier. Temperature was usually not a significant factor in most the top models, although current season average temperature was a component in two of the top three models. For *Ceanothus* a different pattern emerges (Table 1). Most of the top models included variables representing temperature. Moreover, models lacking temperature variables usually were ranked lower than models that included temperature. Three of the

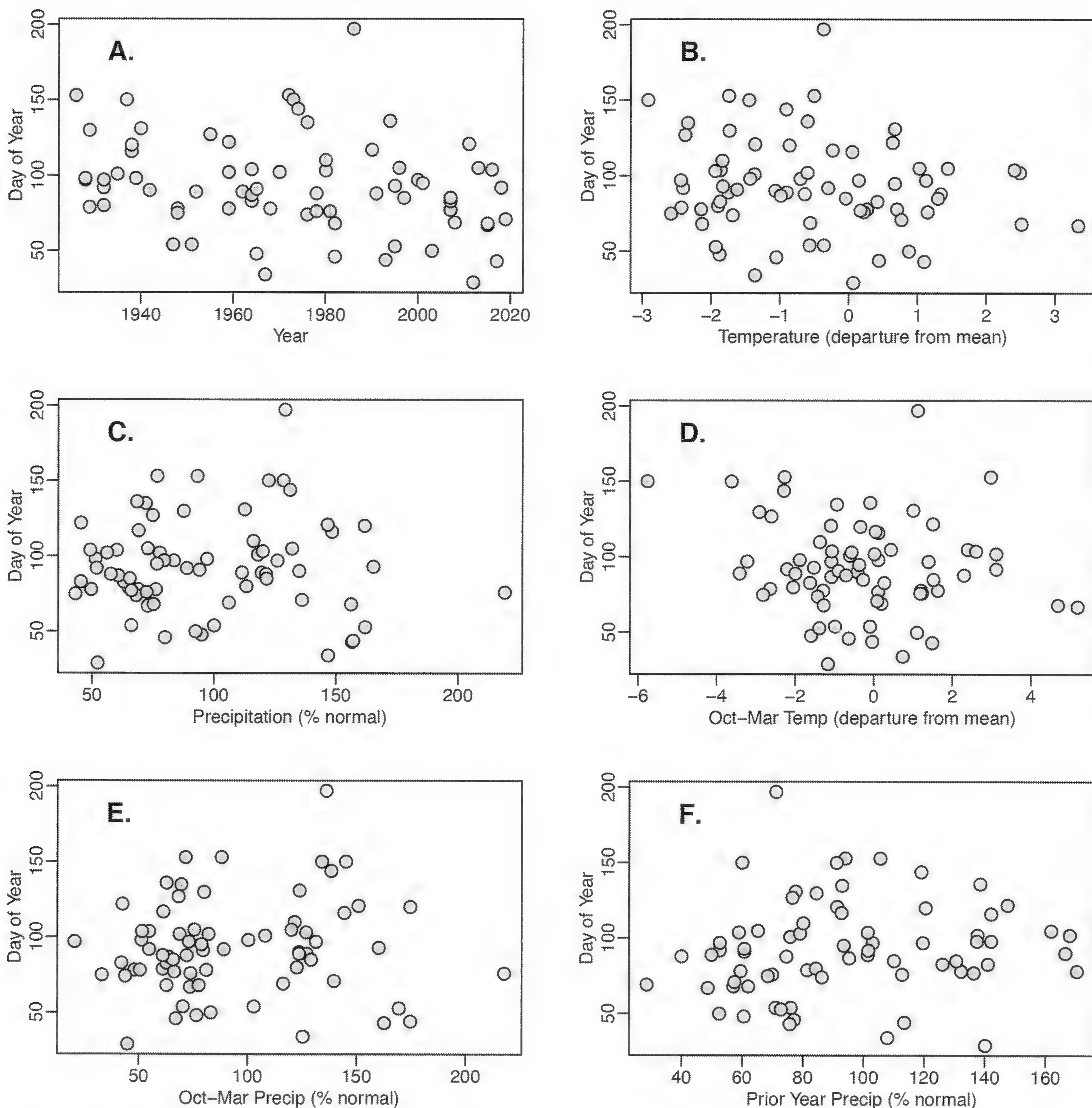


FIG. 4. Day of year flowering for *Ceanothus cuneatus* based on herbarium collections and various climatic factors. Flowering Day of Year versus A) year of collection; B) departure from the mean average temperature for a given year; C) percentage of normal precipitation for a 12-mo period; D) departure from the mean average temperature for a 6-mo period before mean flowering; E) percentage of normal precipitation for a 6-mo period before mean flowering; F) percentage of normal precipitation for a 12-mo period prior to the year of collection.

four highest ranking models incorporated total season average temperature; two included current growing temperature. In this analysis, only one of the four top models included coefficients that only reflected precipitation, that of both the current and previous growing seasons. Examining the coefficients of the best ranked *Ceanothus* model for the flowering date regression indicated two large coefficients (Table 2), with the average temperature for the entire biological year advancing flowering dates, while the current season's average temperature delayed flowering (the current season coefficient was not statis-

tically significant). The balance between variations of these variables would either advance flowering, such as a colder winter and warmer spring, or potentially delay flowering, such as a warm winter and cooler spring to summer. In addition, one of the highest-ranking models included only precipitation from the previous growing season plus precipitation in the month preceding flowering. Other models that were ranked relatively high included both temperature and precipitation values in some combination.

Generalized linear models (GLM's) were applied to the same data set for each species separately

TABLE 1. THE TOP MODELS ASSESSED BY GENERALIZED LINEAR MIXED MODELS (GLMM) FOR FLOWERING IN *ARCTOSTAPHYLOS* AND *CEANOTHUS* SPECIES. Model = variables used in the model; AIC = Akaike information criterion; BIC = Bayesian information criterion. Models indicate the variables most strongly associated with the pattern of flowering phenology as represented in the herbarium collection data. All variables found in each model are indicated. The smallest value for either AIC or BIC represents a higher ranking. In general, models differing by 2–7 AIC units are considered statistically different (Burnham et al. 2011); the other models with larger values not shown. T = growing season (Oct–Sept) temperature departure from normal; P = growing season precipitation as a % of normal precipitation; Prior = previous growing season precipitation as a % of normal precipitation; T5m(T6m) = mean temperature departure from normal for the first 5(6) mo of the growing season (Oct–Feb[Mar]); P5m(P6m) = precipitation as a % of normal for the first 5(6) mo of the growing season (Oct–Feb[Mar]). Normal is calculated as the average of values from 1981–2000 by the National Climate Center.

Taxon	Model	AIC	BIC
<i>Arctostaphylos</i>	P + P5m + Prior + P:P5m + P:Prior + P5m:Prior + Prior:P5m:P	1374.409	1403.681
	P + P5m + Prior + P:P5m + P:Prior + P5m:Prior + Prior:P5m:P + T5m	1376.343	1408.543
<i>Ceanothus</i>	T + T6m	1710.747	1726.514
	T	1711.044	1723.657
	P6m + Prior	1711.044	1723.657
	T + T6m + T:T6m	1711.615	1730.535

(Table 3, Appendix 2). The overall results were similar with some important differences. *Arctostaphylos* species responded more to precipitation variables, while *Ceanothus* species responded more to temperature variables in these analyses as long as the prior season’s precipitation was included. The models indicated some slight differences among the species. *Arctostaphylos andersonii* and *A. hookeri*, both restricted to colder maritime areas, had more high ranked models with responses to temperature variables than *A. glandulosa*. Similarly, both *Ceanothus* species had similar responses to the overall genus models, with the exception that the previous season’s precipitation was found in every high ranked model. As in the GLMM models, coefficients that were significant were both negative and positive, indicating a complex response to these variables (Appendix 2).

DISCUSSION

For these chaparral species in *Arctostaphylos* and *Ceanothus*, flowering phenology has not paralleled historical temperature increases, an aspect of California’s climate that has changed the most over the past century (Figs. 1, 2). Given that these plants are

early flowering species, especially *Arctostaphylos*, they represent divergences from the pattern that has been found in most species (Cleland et al. 2012; Park et al. 2019). Because of the interannual variability inherent in California’s climate, trends in temperature influences on these species may have been constrained by precipitation patterns. For example, patterns of biomass and composition in California’s annual dominated grasslands are strongly influenced by precipitation and temperature interactions (e.g., Pitt and Heady 1978). For these shrub species, both precipitation and temperature variables combine in several ways in predicting the timing of flowering.

In *Arctostaphylos*, the prior year’s total precipitation appeared to have the most frequent influence on flowering, but also this was associated with the current growing season’s precipitation and sometimes temperatures. This pattern is congruent with what other researchers have found in short-term field studies (Baker et al. 1982; Keeley 1987a; Keeley and Keeley 1988). Flowering in *Ceanothus* species may respond to large fluctuations in precipitation between years (Baker et al. 1982; Schlesinger et al. 1982; Keeley 1987a), as indicated in one of the higher-ranking GLMM models and all of the GLM models for *Ceanothus* species. *Ceanothus*, however, for this

TABLE 2. COEFFICIENTS FOR THE TOP GLMM MODEL FOR EACH GENUS. Abbreviations as in Table 1.

Taxon	Model effect	Value	Std. error	DF	t-value	P-value
<i>Arctostaphylos</i>	(Intercept)	−18.091	102.267	128	−0.177	0.859
	P	−1.641	1.365	128	−1.202	0.232
	P5m	5.030	1.133	128	4.438	0.0000
	Prior	2.029	1.106	128	1.835	0.069
	P×P5m	−0.016	0.009	128	−1.816	0.072
	P×Prior	0.016	0.0149	128	1.105	0.271
	P5m×Prior	−0.053	0.012	128	−4.270	0.0000
	P×P5m ×Prior	0.0001	0.0001	128	1.754	0.082
<i>Ceanothus</i>	(Intercept)	143.835	26.700	169	5.387	0.0000
	T	−8.648	3.668	169	−2.357	0.0195
	T6m	3.924	2.603	169	1.507	0.134

TABLE 3. THE TOP FLOWERING MODELS ASSESSED BY GENERALIZED LINEAR MODELS (GLM) FOR *ARCTOSTAPHYLOS* AND *CEANOTHUS* SPECIES. Models indicate the variables most strongly associated with the pattern of flowering phenology as represented in the herbarium collection data. Models are presented ranked with the best models at the top as ranked by AIC. Abbreviations as in Table 1.

Taxon	Model	AIC
<i>Arctostaphylos glandulosa</i>	P + Prior + Prior:P	952.54
	P + Prior + Prior:P + T5m	953.26
	P + Prior + Prior:P + T	954.21
<i>Arctostaphylos andersonii</i>	T + T5m +P5m + P:P5m +T:P + T5m:P + T5m:P5m + T:P:P5m + T5m:P:P5m	354.92
	P5m + T + T5m +T:T5m + TP5m +T6m:P5m +T:T5m:P5m	384.20
<i>Arctostaphylos hookeri</i>	P + P5m + T + T5m + T:T5m + P:P5m +T:P + T:P5m + T5m:P + T5m:P5m +T:P:P5m + T5m:P:P5m +T:T5m:P +T:T5m:P5m +T:T5m:P:P5m	461.17
	P + P5m + T + T5m + P:P5m +T:T5m	475.88
	Prior + P6m + T6m + T6m:P6m	843.77
<i>Ceanothus cuneatus</i>	Prior + T + P6m + T:P6m	846.49
	Prior + T + P6m + Prior:T + Prior:P6m + T:P6m + Prior:T:P6m	846.97
<i>Ceanothus thyrsiflorus</i>	Prior + T + P6m + Prior:T + Prior:P6m + T:P6m + Prior:T:P6m	1740.20
	Prior + T + P6m + T:P6m	1750.10

flowering data set, also responds to temperature variables that in various forms are found in most of the best models predicting flowering date. The coefficients in the models of both genera often conflict in their influence on the timing of flowering suggests a more complicated response to seasonal variation in temperature and precipitation rather than a straightforward advancement of flowering as climate warms. These two genera appear to be responding to an ensemble of influences that interact, so far yielding essentially a lack of net phenological shift. While species from the same genus may respond differently from each other (Gerst et al. 2017), in this case, the few species investigated appear to respond similarly within each genus.

The lack of a flowering phenological response in these species to warmer temperatures over the last century is perhaps related to the marginal and variable precipitation patterns in California’s climate. The models interpreting their responses presented here are unfortunately coarse-grained, and yet they are consistent with other studies (e.g., Baker et al. 1982; Keeley 1987a). In addition, with perennial, woody species, interactions among these environmental factors may also influence the timing of flowering. Plus, the strength of these interactions’ influence on phenological timing may vary among years depending on an individual plant’s physiological condition at the end of the summer rainless period. For example, an extensive drought or large fruit crop in the previous year may limit the amount of photosynthate available for the subsequent year’s flowering regardless of otherwise favorable conditions for flowering, potentially shifting the timing of flower initiation (e.g., Baker et al. 1982; Schlesinger et al. 1982; Keeley 1987a, 1987b; Keeley and Keeley 1988). Keeley (1987a) and Keeley and Keeley (1988) proposed that prior year environmental conditions can influence the timing or abundance of flowering in subsequent

years, especially by limitations on or enhancement of photosynthate, and the subsequent impact on production of dormant flowering buds. The higher-ranking models in this study indicate that prior-year precipitation influences the temporal flowering responses of both genera. Another among-year influence that can influence flower timing is supra-annual variation in fruit production; this is a general pattern in woody plants without dispersal mutualists or with seed predators as dispersal agents (Herrera et al. 1998), as is the case in these two genera (Warzecha and Parker 2014; Parker 2015).

The lack of phenological flowering responses to more than a century of temperature increase suggests phenological emphases in these two lineages that are comparable to those found in other woody plants of semi-arid habitats (Beatley 1974; Law et al. 2000; Preito et al., 2008; Crimmins et al. 2011; Grossiord et al. 2017). Both *A. glandulosa* and *C. cuneatus* are widely distributed and collections from different locations probably have experienced different climatic histories. While the other three taxa are more narrowly distributed and shared a more similar climatic history; their populations, however, are arrayed in their distribution in a variety of soils, soil depth, shading from trees, differential elevation effects of the coastal marine layer, and other factors that vary among populations in coastal areas. Parsing actual population responses would require more focused research, controlling for variation in elevation, topographical aspect, or other factors. These narrower constraints have been found to be significant for some developmental stages in these and other plants (Keeley 1987a, 1987b; Keeley and Keeley 1988; Maclean 2020).

The lack of phenological shifts may also represent a sampling issue: larger numbers of individuals for these species, plus additional species for each genus

may reveal a different pattern. For example, both genera have species above the annual snow level and those species may respond more readily to trends in temperature. This limited study suggests the predicted increase in climatic variability in California will likely continue to constrain simple climatic responses in lower elevation woody taxa in the future (Gordo and Sanz 2010). Given the differential influences in their models, *Ceanothus* species may respond to future temperature warming well before *Arctostaphylos* species; *Ceanothus* species, for example, may have already started responding, but that response was statistically undetected due to relatively few collections over the last 20 yr. Future studies also may need to incorporate experimental conditions varying temperature and soil moisture patterns in common garden situations to determine how physiological conditions influence flowering in these genera in the context of varying combinations of temperature and precipitation.

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APPENDIX 1

THE FOLLOWING ARE THE SPECIMENS USED IN DETERMINING PHENOLOGICAL STATES. THE SPECIFIC HERBARIA ARE LISTED USING THEIR ABBREVIATIONS.

Arctostaphylos glandulosa subsp. *glandulosa*: CAS27405, CAS27195, CAS38856, CAS38778, CAS507644, CAS27204, CAS38842, CAS38855, CAS179647, CAS179649, CAS216564, CAS179652, CAS179654, CAS179655, CAS164580, CAS163646, CAS163641, CAS163639, CAS201940, CAS171306, CAS171207, CAS178407, CAS194674, CAS504664, CAS162244, CAS189254, CAS200532, CAS193593, CAS186157, CAS188675, CAS164647, CAS228416, CAS216316, CAS216487, CAS239247, CAS239248, CAS269558, CAS268411, CAS475648, CAS242753, CAS269574, CAS269572, CAS318636, CAS371643, CAS371646, CAS374764, CAS374765, CAS385987, CAS385985, CAS394056, CAS475925, CAS475631, CAS994592, CAS493305, CAS475633, CAS484548, CAS557007, CAS1046547, CAS1046492, CAS1046546, CAS618248, CAS801083, CAS800138, CAS800128, CAS743271, CAS658266, CAS658264, CAS658267, CAS740374, CAS734062, CAS734050, CAS742090, CAS1007699, CAS951805; *A. andersonii*: CAS49392, CAS91950, CAS214744, CAS86763, CAS86796, CAS86776, CAS86778, CAS392350, CAS392357, CAS122344, CAS212879, CAS504920, CAS202229, CAS219965, CAS301728, CAS219972, CAS219963, CAS219962, CAS220011, CAS355188, CAS392352, CAS392347, CAS392356, CAS557251, CAS392349, CAS557259, CAS557257, CAS475932, CAS621546, CAS731140, Parker&Vasey 119, Parker&Vasey 177, Parker&Vasey 464, Parker&Vasey 864, Parker&Vasey 866, Parker&Vasey 863; *A. hookeri*: CAS91948, CAS91858, CAS466406, CAS27445, CAS88044, CAS27447, CAS141901, CAS141428, CAS138889, CAS134670, CAS38907, CAS557263, CAS17891, CAS13756, CAS38905, CAS194680, CAS74440, CAS220015, CAS301730, CAS239213, CAS475420, CAS382185, CAS358577, CAS475410, CAS402849, CAS557274, CAS475415, CAS475416, CAS475417, CAS475419, CAS475418, CAS740366, Parker&Vasey 88, Parker&Vasey 89, Parker&Vasey 86, Parker&Vasey 242, Parker&Vasey 555, Parker&Vasey 870; *Ceanothus cuneatus*: AHUC102187, AHUC102188, CAS:-BOT-BC:32726, CHSC055268, CHSC064367,

CHSC077440, CHSC090088, DAV304685, DAV304523, DAV304524, DAV304526, DAV304527, DAV304529, DAV304533, DAV304535, DAV304542, DAV304543, DAV304544, DAV304547, DAV304549, DAV304551, DAV304552, DAV304554, DAV304561, DAV304607, DAV304608, DAV304611, DAV304615, DAV304617, DAV304622, DAV304634, DAV304639, DAV304645, DAV304651, DAV304652, DAV304670, DAV304678, DAV304684, DAV324542, DAV324543, DAV324545, DAV392077, LOB110753, LOB110752, LOB110762, OBI124019, OBI161895, OBI161908, OBI161909, OBI161910, RSA0170332, RSA0208758, RSA0215365, SBBG167484, SBBG167491, SBBG167494, SBBG169632, SBBG169635, SBBG169649, SBBG169654, SBBG169656, SBBG171548, SBBG171564, SBBG171570, UCSB016098, UCSB016100, UCSB016108, UCSB016116, UCSB016152, UCSB016158, UCSB052313; *Ceanothus thyrsiflorus*: SBBG169845, SBBG171199, OBI124438, LOB110808, SBBG168217, SBBG172582, UC18432, UC1281280, OBI124443, SBBG171174, OBI124409, SBBG171203, UCSB016534, SFV112236, OBI124446, SBBG167821, UCSB016535, UCSC100004896, SBBG169847, UC1071409, SBBG172584, OBI124459, OBI124452, SBBG171173, SBBG171178, CSLA014530, UC1505527, SDSU12875, SBBG171168, DAV304796, LOB110804, LOB110805, SJSU5720, OBI124426, UCSC100004888, SFV112237, SBBG171183, SDSU10424, LOB110807, SFV112235, OBI124428, OBI124430, OBI124455, UCSB016530, UCSB016531, OBI124457, SBBG171184, SBBG169837, CSLA014458, MACF032735, UCSB016517, UCSC100004905, UCSC100004917, DAV304784, SBBG171211, UCSB016528, OBI124434, DAV304792, SJSU5728, UCD36556, UCD112730, UCSC100004890, UCSC100004886, UCSC100004887, SFV112233, SBBG171153, DAV304787, OBI124449, SBBG171152, UCSC100004889, SFV112238, UCSC100004916, DAV304782, SBBG169840, UCSC100004909, SBBG171200, DAV304786, UCSC100004912, SBBG169152, OBI124431, SBBG171204, UCSC100004914, SBBG171177, DAV304793, UCSC100004910, UCSC100004915, UCSC100004898, DAV304824, DAV304785, SBBG168223, AHUC102291, SBBG168222, CSLA014532, UCSC100004897, SDSU10425, UCSB016526, SBBG168213, DAV304823, CSLA014538, SBBG171207, SBBG169937, DAV304789, UCSC100004834, SBBG172593.

APPENDIX 2
COEFFICIENTS FOR THE TOP GLM MODEL FOR EACH SPECIES.
Abbreviations as in Table 1 in the main text. The colon separating variables indicates interactions between or among those variables. Asterisks indicate degree of statistical significance: * = 0.05; ** = 0.005; *** = 0.0005.

Species	Coefficient	Estimate	Std. Error	z value	P value
<i>Arctostaphylos glandulosa</i>	(Intercept)	5.425e+00	1.068e−01	50.789	<2e−16 ***
	P	−3.899e−03	1.020e−03	−3.822	0.000133 ***
	Prior	−2.406e−03	1.103e−03	−2.181	0.029198 *
	P:Prior	3.500e−05	1.101e−05	3.179	0.001476 **
<i>Arctostaphylos andersonii</i>	(Intercept)	3.763e+00	4.500e−01	8.364	<2e−16 ***
	T	3.351e+00	9.059e−01	3.699	0.000217 ***
	T5m	−8.553e+00	2.253e+00	−3.797	0.000147 ***
	P	1.848e−03	5.838e−03	0.317	0.751574
	P5m	3.137e−02	1.309e−02	2.397	0.016538 *
	T:T5m	−2.198e+00	1.420e+00	−1.548	0.121722
	P:P5m	−2.124e−04	7.353e−05	−2.888	0.003878 **
	T:P	−4.621e−02	1.231e−02	−3.753	0.000175 ***
	T:P5m	−1.481e−02	1.447e−02	−1.024	0.306071
	T5m:P	6.847e−02	2.032e−02	3.370	0.000752 ***
	T5m:P5m	1.092e−01	3.281e−02	3.330	0.000869 ***
	T:P:P5m	2.677e−04	1.131e−04	2.366	0.017962 *
	T5m:P:P5m	−9.273e−04	2.522e−04	−3.677	0.000236 ***
	T:T5m:P	6.256e−03	7.888e−03	0.793	0.427697
	T:T5m:P5m	4.393e−02	2.393e−02	1.836	0.066362 .
	T:T5m:P:P5m	−2.906e−04	1.672e−04	−1.738	0.082202 .
<i>Arctostaphylos hookeri</i>	(Intercept)	5.774e+00	1.242e−01	46.500	<−2e−16 ***
	T	−1.118e−01	2.383e−02	−4.690	2.73e−06 ***
	T5m	4.126e−02	2.236e−02	1.845	0.065 .
	P	−9.797e−03	1.526e−03	−6.422	1.35e−10 ***
	P5m	−2.890e−03	1.572e−03	−1.838	0.066 .
	T:T5m	−1.101e−02	9.455e−03	−1.165	0.244
	P:P5m	6.825e−0	5 1.188e−05	5.743	9.31e−09 ***
<i>Ceanothus cuneatus</i>	(Intercept)	5.1233020	0.0354029	144.714	<2e−16 ***
	T6m	0.0325149	0.0127056	2.559	0.01049 *
	P6m	0.0001846	0.0002231	0.827	0.40798
	Prior	0.0007372	0.0002610	2.824	0.00474 **
	T6m:P6m	−0.0004966	0.0001234	−4.024	5.71e−05 ***
<i>Ceanothus thyrsiflorus</i>	(Intercept)	4.551e+00	1.226e−01	37.129	<2e−16 ***
	T	−2.939e−01	1.139e−01	−2.579	0.009895 **
	P6m	3.505e−03	1.382e−03	2.536	0.011218 *
	Prior	2.486e−03	1.215e−03	2.046	0.040725 *
	T:P6m	3.258e−03	1.332e−03	2.445	0.014471 *
	T:Prior	3.865e−03	1.138e−03	3.398	0.000680 ***
	P6m:Prior	−4.897e−05	1.365e−05	−3.588	0.000334 ***
	T:P6m:Prior	−4.805e−05	1.301e−05	−3.694	0.000221 ***

DOMINANT SONORAN DESERT PLANT SPECIES HAVE DIVERGENT
PHENOLOGICAL RESPONSES TO CLIMATE CHANGE

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ABSTRACT

The southwestern U.S. is a global hotspot of climate change. Models project that temperatures will continue to rise through the end of the 21st century, accompanied by significant changes to the hydrological cycle. Within the Sonoran Desert, a limited number of studies have documented climate change impacts on the phenology of native plant species. Much of this phenological work to understand climate change impacts to phenology builds on research conducted nearly three decades ago to define flowering triggers and developmental requirements for native keystone Sonoran Desert woody species. Here we expand on the drivers and explore recent phenological trends for six species using a unique 36-year observational data set. We use statistical models to determine which aspects of climate influence the probability of flowering, and how flowering time may respond to climate change. We move beyond traditional models of phenology by incorporating different metrics of moisture availability in addition to temperature, weather, and climate at several time scales, including daily, weekly, seasonal, and antecedent conditions. Our results provide evidence of a trend towards earlier flowering (on the order of 1–4 days per decade) for five of the six species analyzed, and no trend for one species. The species we evaluated had contrasting phenological responses to different aspects of climate, suggesting individualistic changes in phenology and the potential of divergent plant community flowering patterns under future climate change. Understanding recent changes in flowering phenology and their climatic triggers is important to anticipating whether plant species can attract pollinators, reproduce, and persist within the community under continued climate change.

Key Words: arid ecosystems, climate change, daily weather, flowering, phenology, plant reproduction, Sonoran Desert, vapor pressure deficit.

Phenology, the timing of biological events, from bird migrations to flowering, has become a ‘leading indicator’ of climate change impacts (Parmesan and Yohe 2003). Climate-induced shifts in phenology can negatively affect individual fitness if the new timing of a biological event does not correspond to when environmental conditions are optimal (Willis et al. 2008). Changes in the timing of growth and reproduction may ultimately be necessary to track the rapid pace of climate change (Cleland et al. 2012). Evaluations of how climate and phenology are coupled, and how they have changed through time, can provide insight on the potential for organisms to persist under climate change. Understanding whether species respond in similar or divergent ways to different aspects of climate is essential to understanding future community composition, trophic dynamics, and ecosystem function. Decades of phenological research has demonstrated the practicality and utility of linking ground-based phenological and meteorological measurements. Many of these past studies have focused primarily

on temperature and the accumulation of growing degree days as phenological drivers (e.g., Basler 2016). This research has demonstrated that warming temperatures and a more rapid accumulation of growing degree days (AGDD) have accelerated the phenology of many species. Growing degree days provide a measure of heat accumulation in the spring, defined as the number of degrees by which average daily temperatures exceed some baseline (e.g., freezing). They are accumulated (summed) daily following a predefined start date. AGDD has proven to be an effective predictor of phenological transitions in plant, insect, and other animal species (Cayton et al. 2015; Crimmins et al. 2017) leading to the development of gridded phenological indices that have been used to forecast and map the onset of spring (Schwartz 1997; Schwartz et al. 2013; Ault et al. 2015) and, more recently, species-level phenological forecasts (Taylor and White 2020). However, multiple aspects of plant biology, from phenology to growth and mortality, are also influenced by the balance between the timing and magnitude of

precipitation and atmospheric demand for moisture (e.g., Novick et al. 2016; Choat et al. 2018). Understanding the windows of time in which precipitation, atmospheric demand for moisture, and temperature-related variables (e.g., AGDD) affect plant phenology is essential, and can reveal how changes in moisture availability mediated by climate change may influence ecosystems.

Although temperature-based approaches to phenology have been successful, a rich history of phenological research has shown that rainfall and moisture availability also play an important role in the timing of phenological events from tropical rainforests to the water-limited Sonoran Desert of North America (Gentry 1974; Reich and Borchert 1984; Ashton et al. 1988; Fox 1990; Bowers and Dimmitt 1994; Lasky et al. 2015). Nearly three decades ago, Bowers and Dimmitt (1994) showed that the flowering of six dominant woody species is triggered by rain and photoperiod, after sufficient accumulations of growing degree days. Since that time, the role of rainfall has received increasing attention in phenological research in dryland ecosystems (Mazer et al. 2015; Park and Mazer 2018; Renzi et al. 2019; Elmendorf et al. 2019). Another important aspect of moisture availability is the “thirst” of the atmosphere, or atmospheric demand. The role of atmospheric demand is only now beginning to appear in the phenological literature, but recent research has shown that increases in atmospheric demand can alter, often delaying, plant phenology (Adams et al. 2015; Wion et al. 2020).

Many of the climatic factors that influence plant performance are rapidly changing in the southwestern U.S. Temperatures across the Sonoran Desert have risen by 1–3°C in the last century (Munson et al. 2012) and the southwestern U.S. is expected to rise 3–6°C by 2100 (IPCC 2013). This warming has extended the length of the frost-free season (Osland et al. 2021) and – in conjunction with lower relative humidity – has been associated with prolonged drought conditions since 2000 (Weiss and Overpeck 2005; Weiss et al. 2009). Indeed, in the southwestern U.S., temperature and vapor pressure deficits (VPD) are increasing faster than in other regions (Driscoll et al. 2020). In the Mojave Desert, for example, there have been substantial increases in both mean annual temperature and mean daily maximum VPD, alongside decreases in total annual precipitation, resulting in much lower ecologically available water (Driscoll et al. 2020). In general, there is broad consensus that aridity will continue to increase in the Desert Southwest due to both decreasing precipitation and increasing atmospheric demand as we move into the mid to late 21st century (Seager and Vecchi 2010; Cayan et al. 2010; Udall and Overpeck 2017).

How will phenology in the Desert Southwest respond to recent and ongoing climate change? Bowers and Dimmitt (1994) showed that six dominant woody species in the Sonoran Desert have a spectrum of phenological strategies that vary in their

relation to photoperiod, temperature, and rainfall. These findings are part of a growing body of research demonstrating variation in phenological responses to climate change across species (Fitter and Fitter 2002; Parmesan 2007; Sherry et al. 2007; Crimmins et al. 2010; CaraDonna et al. 2014; CaraDonna and Inouye 2015). Here, we revisit the work of Bowers and Dimmitt (1994), focusing on many of the same species, but with a more complete phenology record and new phenological modeling techniques. With this approach, our objectives were to determine (1) how spring flowering time has changed over the last three decades, (2) which aspects of climate have influenced the probability and timing of flowering, and (3) how climatic factors that have influenced flowering phenology historically are forecasted to change into the future in the Sonoran Desert. We focus our attention on spring flowering time because of the potential for phenological advances due to warming, phenological triggers due to moisture availability from cool-season frontal storms, and the higher consistency of floral output and pollinator activity across species at this time of year. The climatic factors we focused on were selected because they are biologically meaningful drivers of flowering phenology in the Sonoran Desert, and include temperature variables (growing degree days, vapor pressure deficit, and number of freezes) and precipitation variables at multiple time scales (2-week rainfall, number of days since rainfall, antecedent seasonal rainfall, and the Pacific Decadal Oscillation Index). We contextualize our phenological modeling results with existing climate projections to understand how the patterns we observed might play out to the end of the century.

METHODS

Study Area

The study site in the Tucson Mountains (15 km west of Tucson, 32.2434°, –111.1672°) of southern Arizona straddles the boundary of Tucson Mountain Park, a 20,000 acre county-managed park, and the Tucson Mountain District of Saguaro National Park. Within the boundaries of Tucson Mountain Park sits the Arizona-Sonora Desert Museum (Desert Museum), a zoo, botanical garden, museum, and research facility maintaining living collections of native Sonoran Desert plants and animals. Phenological observations were recorded on site and in adjacent desert habitat at elevations ranging from 850 m to 1000 m. The 50 hectare study area includes a south-facing bajada, a dry bedrock canyon (King Canyon), and the adjacent steep slopes of this canyon. The south-facing bajada is a thin layer (20–50 cm) of rocky soil underlain by caliche (alluvium cemented by calcium carbonate). The bedrock is Mesozoic mudstone and Cenozoic era breccia of shale and volcanics known as the Tucson Mountain Chaos

(National Park Service 2020). Steep slopes are a mixture of this breccia and loose rock and soil.

The vegetation type in the study area is Arizona Upland Sonoran Desertscrub (Shreve 1951; Turner and Brown 1982). Dominant species include trees (*Parkinsonia microphylla* Torr., Foothill Palo Verde; *Olneya tesota* A.Gray, Desert Ironwood) shrubs (*Simmondsia chinensis* (Link) C.K.Schneid., Jojoba; *Fouquieria splendens* Engelm., Ocotillo; *Vachellia constricta* (Benth.) Seigler & Ebinger, Whitethorn Acacia; *Larrea tridentata* (DC.) Coville, Creosote Bush; *Encelia farinosa* A.Gray ex Torr., Brittlebush; and *Ambrosia deltoidea* (Torr.) W.W.Payne, Triangle-leaf Bursage), and cacti (*Carnegiea gigantea* (Engelm.) Britton & Rose, Saguaro; *Opuntia* spp., Prickly Pears; *Cylindropuntia* spp., Chollas).

The climate in the study area is arid with a bimodal precipitation regime. Approximately half of the annual rainfall arrives in often high-intensity summer North American Monsoon storms (early July–September) and half in larger, slow-moving storms during the fall and winter (October–March). April, May, and June are often without rain. The average annual rainfall at the Desert Museum during the period of study was 336 mm (estimated with Daymet V3; Thornton et al. 2016). Maximum temperatures in summer frequently exceeded 40°C. Average winter minimum temperature during the period of study was –6.7°C at the Desert Museum with an annual average daily mean temperature of 20.0°C.

To contextualize our phenological modeling results with expectations of how climate is projected to change in the study area, we used forecasts from an existing data product that integrates 20 climate models from the “high emissions” RCP8.5 scenario for rainfall, VPD, and minimum and maximum temperature. We averaged the minimum and maximum temperature estimates to obtain an estimate of mean temperature. These existing data are available from the Climate Toolbox (<https://climatetoolbox.org/>) at a ~4 km resolution across the U.S. (Abatzoglou and Brown 2012; Abatzoglou 2013). We also extracted historical climate data from the Climate Toolbox at the same ~4 km resolution to depict future climate projections alongside historical trends at the same spatial resolution.

Phenological Observations

Between 1982 and 2018, staff from the Desert Museum’s Botany Department recorded phenological observations on plant populations on the grounds of the Desert Museum, on the adjacent south-facing bajada, and in King Canyon. Observations were collected up to four times per month for a total of up to 48 observations per year. Not all species were sampled every year. Importantly, our model (explained below) can accommodate missing observations. Staff recorded their phenological observations for populations rather than individuals. The number

of individuals within a population ranged from approximately 30 (*V. constricta*) to over 100 (*A. deltoidea*). Although observations of multiple phenophases were recorded (budding, flowering, fruiting, leafing out, and leaf fall), here we report only on the onset of first flowering. To avoid the potential confounding effect of supplemental watering of specimens on Museum grounds, we used phenological observations only from the unmanaged King Canyon site. Although herbarium specimens are increasingly used in phenological research (Willis et al. 2017; Jones and Daehler 2018; Pearson et al. 2021), these observations are only associated with a single date, and are highly left censored (meaning that it is only known that flowering occurred some time before the observation date), ultimately containing much less information than direct and regular observations of phenological transitions, such as the weekly observations made by Desert Museum staff. Because of this issue, in combination with a low number of flowering herbarium specimens from the Desert Museum grounds, we limit our phenological observations to the direct and regular observations of live, naturally occurring plants by Desert Museum staff.

Study Species

We examined the flowering phenology of six dominant drought-deciduous woody perennials in the Arizona Upland subdivision of the Sonoran Desert for which we had an abundance of phenological data (Table 1). Drought-deciduous shrubs and trees represent an important functional group within warm deserts (Shreve 1951). Although as a group they have adopted the drought-evading strategy of shedding leaves in response to increasing water stress, the morphological and physiological diversity within this functional group is immense and has resulted in a large diversity of phenological strategies.

Two of our species, *Parkinsonia microphylla* (Fabaceae) and *Olneya tesota* (Fabaceae) are trees, thought to be deep-rooted (Cannon 1911; Phillips 1963; Canadell et al. 1996), which flower between April–June. While *O. tesota* drops its leaves only once per year, just as flower buds appear, *P. microphylla* can remain leafless most of the year by relying on its photosynthetic trunk and branches (Smith 1997).

Vachellia constricta (Fabaceae), Whitethorn Acacia, is a drought-deciduous shrub, sometimes reaching the stature of a small tree, which blooms in late spring (May–June) and again in summer/autumn (July–October) (McGinnies 1983). It is the only one of our species which has evolved to take advantage of the bimodal distribution of rainfall in the Sonoran Desert by flowering in both the spring and summer.

Fouquieria splendens (Fouquieriaceae), Ocotillo, is a drought-deciduous, stem-succulent shrub (Killingbeck 2019), the only one of our species to have

TABLE 1. PHENOLOGICAL WINDOWS AND FUNCTIONAL TRAITS OF FOCAL SPECIES. The start date (mm/dd) marks the beginning of the window within which we might expect to see flowering. The end date (mm/dd) is used to ensure that monsoon season events, which occur after phenological events during the usual cool-season period, are not accidentally included in the model. Our focus throughout is on “cool” (winter/spring) flowering events as opposed to monsoon season events. For context, we also provide additional growth habit and life history information.

Species	Start	End	Growth form and leaf traits	Dominant pollinators
<i>O. tesota</i>	3/1	7/31	evergreen tree	bees
<i>P. microphylla</i>	3/1	7/31	stem photo-synthetic drought-deciduous tree	bees
<i>V. constricta</i>	2/1	6/30	drought deciduous shrub/tree	bees
<i>F. splendens</i>	12/1	6/30	drought deciduous semi-succulent shrub	hummingbirds and bees
<i>A. deltoidea</i>	12/1	6/30	drought deciduous shrub	wind
<i>E. farinosa</i>	10/1	6/30	drought-deciduous shrub	butterflies and/or flies

adopted both deciduousness and stem succulence in response to highly limited and highly variable water availability. In the Sonoran Desert, it blooms primarily in spring (March–May; Waser 1979), but massive autumn flowering can occur under some circumstances (Felger 1980), and winter (January–February) flowering is possible.

Encelia farinosa (Asteraceae), Brittlebush, and *Ambrosia deltoidea* (Asteraceae), Triangle-leaf Bursage, are drought-deciduous semi-woody subshrubs. *Encelia farinosa* blooms mainly in spring (February–May), and given enough rain and lack of frost, from October–January as well. *Ambrosia deltoidea* is one of the most abundant species in the Arizona Upland subdivision of the Sonoran Desert. It typically flowers from February through early April. This is the only wind-pollinated species within our focal taxa.

Of the original six species in Bowers and Dimmitt (1994) we excluded *Larrea tridentata*, Creosote Bush, which was found in flower in every month of the year during the 36 years of phenology observations collected at our study site. We sought to model the onset of cool (winter/spring) season flowering for each species, but defining a consistent cool season flowering phenology window for *L. tridentata* proved difficult and cast doubt on our ability to produce reliable results for this species.

Model and Covariates

Our models of phenological transitions included weather variables as drivers, which we calculated using Daymet, a 1 km resolution gridded data product that provides estimates of daily weather parameters, including minimum and maximum temperature, precipitation, day length, and the partial pressure of water vapor (Daymet V3; Thornton et al. 2016). Daymet data were obtained using Google Earth Engine (Gorelick et al. 2017). We developed precipitation-based covariates to consider various time scales including rolling 2-week rainfall sums, the number of days since the most recent rainfall event (≥ 5 mm; hereafter ‘rainless days’), and antecedent rainfall – i.e., total precipitation during the monsoon season (June 15–September 30) preceding the early, cool-season flowering that we focused

on modeling in this study. We developed temperature-based covariates as accumulated growing degree days (over a 10°C baseline), and the number of freezes (the total number of days with minimum temperatures below 28°F (-2.22°C), a threshold commonly used to define “hard” freezes). We developed variables to represent vapor pressure deficit as a 2-week rolling mean. We derived VPD from Daymet’s partial pressure of water vapor (E_a) by first calculating saturated vapor pressure (E_s) with the appropriate Arden Buck equation (Buck 1981), using the formula

$$E_s = 6.116441 \times 100 \times 10^{(7.591386 \times T)/(240.7263+T)}$$

where T is the mean of minimum and maximum temperature in degrees Celsius. We then calculated VPD as $E_s - E_a$. Finally, we include variables to represent a large-scale climate oscillation that controls much of the decadal-scale variation in moisture in the region, the Pacific Decadal Oscillation (PDO) with the PDO Index (Zhang et al. 1997; Mantua et al. 1997).

We modeled daily probabilities of flowering and, by extension, the distribution of flowering times, for each of these species using covariates representing current and antecedent temperature, precipitation, and vapor pressure deficit, and the PDO index. All modeling was performed using the R package *tempo* (Landau and Zachmann 2019). The landing page for the software provides a mathematical description of the model. This Bayesian model predicts the occurrence of phenological state transitions (e.g., from non-flowering to flowering) while accommodating censoring, a circumstance in which the value of an observation is only partially known. Phenological data sets are often censored because the event of interest is known only to have occurred sometime between visits to a site, but the precise time of the event is unknown. This is often the case in phenological observations, where technicians may only be able to visit sampling sites intermittently, for example, once per week. The Bayesian model accounts for censoring by imputing event (i.e., flowering) times between bounds provided by – in our case, approximately weekly – visitation dates,

and avoids the bias encountered when censoring is ignored.

Another important feature of the model is that it estimates phenological transitions on a daily basis, which allows us to represent temporally heterogeneous transition probabilities and their drivers. In other words, the chances an event is observed can increase when conditions are favorable, and subsequently decrease, at multiple times during the growing season. The shift in focus from modeling event time to a model of event occurrence has several important consequences (Clark et al. 2014; Diez et al. 2014; Elmendorf et al. 2019). One important strength of such an approach is that environmental variability is allowed to enter a model of event occurrence in an ecologically sensible way. For our data set, the complete daily history of rainfall events enters the model instead of seasonal or annual rainfall averages, which allows the probability of flowering to rise and fall over time. In contrast, models of event times require collapsing environmental variability into a single measure – a single summary measure per event, which ultimately results in less information entering model predictions. These models also do not allow for predictions of daily flowering probabilities, and by extension, they do not allow predictions of the probability that flowering will occur within a given time window. The model we used, however, does accommodate these sorts of predictions.

A bimodal precipitation regime has driven the evolution of two flowering seasons in the Sonoran Desert, with a cool (winter/spring) season and a warm (summer) season. We sought to model first flowering for each species during the cool (winter/spring) season. All time-to-event measures, as well as covariates, were developed using a phenological window that was unique to each species (Table 1). The phenological window for each species spanned the earliest to latest date at which onset of cool season flowering could conceivably occur. For instance, because cool season flowering for *E. farinosa* can occur as early as October, its phenological window spanned the transition between calendar years.

We ran a series of models, which all include AGDD and a quadratic term for AGDD, which allowed the probability of an event to increase and subsequently decrease within a growing season, as opposed to simply monotonically increasing. In addition to AGDD, we included rolling 2-week total precipitation (e.g., the value for a given day is the total precipitation that occurred in the preceding two weeks) and rolling 2-week mean vapor pressure deficit by default. We formed the rest of the models by including all possible combinations of the remaining covariates — number of freezes, rainless days, antecedent monsoonal precipitation, and the PDO index. All models were run with sufficient MCMC iterations to ensure convergence and appropriate characterization of posterior distributions. We used vague priors for all model parameters. We

selected the “best” model for each species using the deviance information criterion (DIC; Spiegelhalter et al. 2002), excluding from consideration models that did not pass posterior predictive checks (Conn et al. 2018). We characterized trend by predicting mean flowering time for each year, followed by a regression of expected flowering times on year for each species.

We visualized the sensitivity to climate and the relative importance of each covariate in the “best” model in two ways. First, we shifted each covariate up or down to $\pm 30\%$ of its observed range, while keeping all other covariates at their daily means, to measure the average departure of phenological transition time from the grand mean resulting from changes to covariates. Evaluating predictions conditional on covariates over some range (their native range or increases or decreases in their extremes) is a useful approach for understanding the relative importance of each covariate in a model. Second, we considered the two years representing the extreme ends of each covariate for each species in our historical record, with all other covariates held at their daily means, to visualize the effect on the distribution of flowering times.

RESULTS

Climate conditions around the Arizona-Sonora Desert Museum are projected to change markedly by 2100 (Fig. 1). Gridded climate products show that mean temperature has been steadily rising in the historical record, and by 2100, is projected by the high-emissions RCP 8.5 scenario to be nearly 27°C , which is almost 6°C warmer than mean temperatures in the region in the 1980s (Fig. 1A). Annual rainfall has been declining historically, from ~ 320 mm in the 1980s to ~ 260 mm in the 2010s, however future projections range from either an increase of ~ 280 mm or a decrease of ~ 120 mm compared to the 1980s (Fig. 1B). The mean of future projections suggests a future average of 310 mm of annual rainfall, and thus little change from the 1980s. Like temperature, vapor pressure deficit has also risen in the historical period, and future projections show a clear rise in VPD, from just over 2 kPa in the 1980s to a range across all models of 2.5–3.5 kPa by 2100 (Fig. 1C). There is strong agreement across climate models for an increase of >1 kPa in VPD in this region (Ficklin and Novick 2017). These historical trends in annual summaries were reflected in the daily or weekly covariate data used in this study. For example, the actual AGDD data for each day of year for every year (1980–2019, Fig. 2), showed more rapidly accumulating growing degrees early in the year and overall higher accumulations by the end of the year in more recent years. Data at this resolution – days, weeks, or months – gave us an ability to model intra-annual phenological forcings with much greater precision, while still reflecting the same overall trends in climate change visible in the annual summaries.

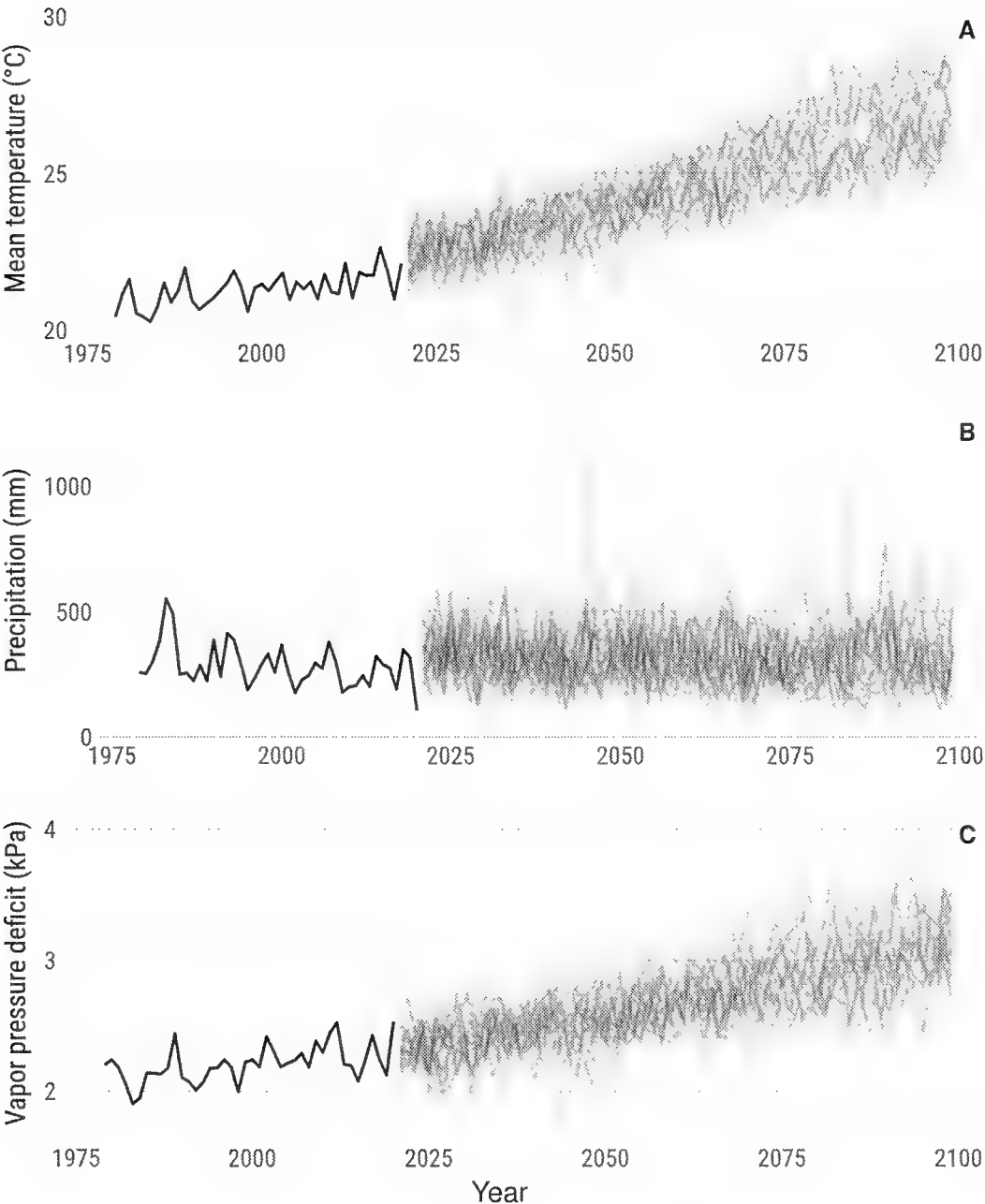


FIG. 1. Annual summaries of historical and projected future climate conditions for the grid cell surrounding the Arizona-Sonora Desert Museum (32.2434°, -111.1672°). Gridded historical and future climate data were retrieved from the Climate Toolbox. Historical data are from the gridded surface meteorological data set, gridMET, which covers the contiguous U.S. at a 4 km spatial resolution from 1979–present. Projected future climate conditions include data from 20 climate models (the semi-opaque lines extending from 2020 to 2100) and one scenario (RCP 8.5) downscaled to a ~4 km resolution across the U.S. for compatibility with the gridMET data using MACAv2-METDATA, version 2 (Abatzoglou and Brown 2012). The three panels show mean annual temperature (the average of maximum and minimum annual temperatures) (A), total annual precipitation (B), and mean vapor pressure deficit (C).

Mean dates of flowering onset varied across the species in our study, from January 28th in *E. farinosa*, to May 9th in *O. tesota* (Table 2). Across our study period, five of six species appeared to exhibit an advance in the onset of flowering, ranging

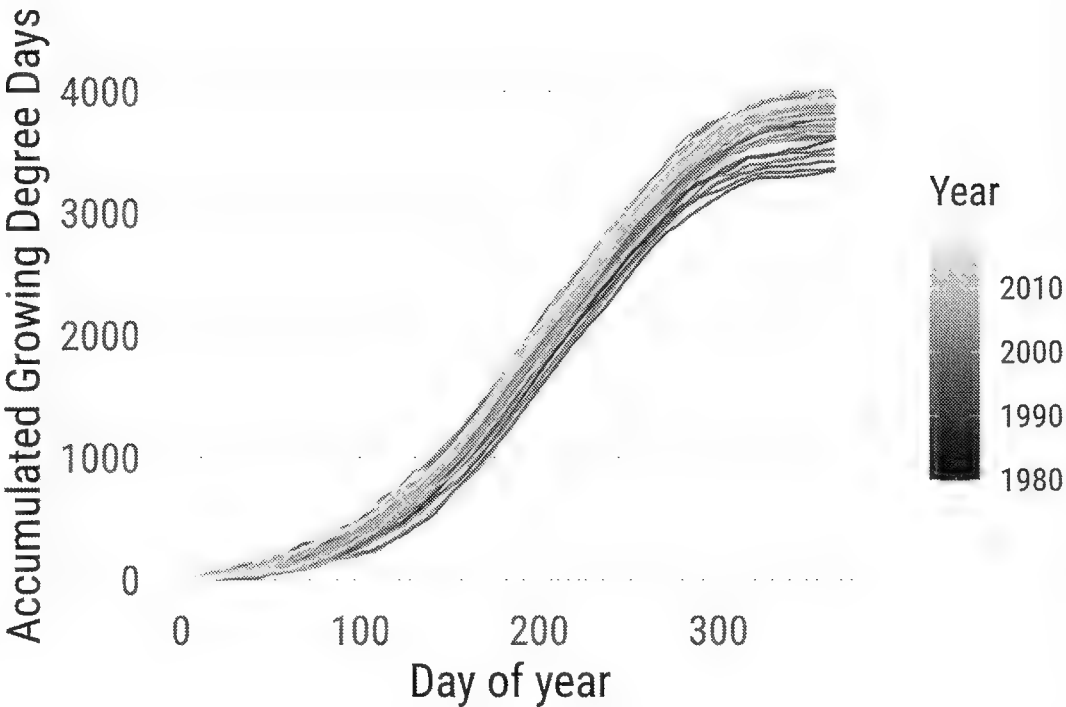


FIG. 2. Accumulated Growing Degree Day (AGDD) data for each day of year for every year (1980–2019) at the Arizona-Sonora Desert Museum, computed using temperature information from Daymet. These daily measures provide an example of the type of climate/weather variable used in the phenological models developed for each species. The AGDD summaries seen here were computed from Jan 1 to Dec 31 of each year using a 10 °C baseline temperature.

from 1.05 to 4.39 days earlier per decade (Table 2, Fig. 3, Table S1). One species, *E. farinosa*, showed an average predicted advance of 0.54 days per decade, but with high uncertainty. Our model predicted a ~42% probability that *E. farinosa* was actually experiencing phenological delay over time (Table 2, Fig. 3, Table S1). The pattern of phenological advance seen among the rest of the species was more certain, with chances of phenological advance ranging from ~84% for *V. constricta* to 100% for *O. tesota* and *A. deltoidea* (Table 2).

Phenological models showed that the effect of AGDD, and its square (AGDD raised to the second power, which is done to model quadratic effects), were the most important factors for the onset of flowering across all species, with large coefficients (Fig. 4, Fig. S1). All species showed an advancing trend (earlier flowering) with increasing AGDD (Table 3, Fig. 4). Rolling 2-week rainfall advanced the flowering of *P. microphylla* and *F. splendens* and delayed the flowering of the remaining four keystone species (Table 3, Fig. S1). Rolling two-week mean

TABLE 2. SUMMARIES OF PHENOLOGICAL TRENDS ACCORDING TO THE TREND LINES IN FIG. 3. Trends were estimated using a truncated normal linear regression of mean flowering onset dates on year. The expected mean flowering time in 1982, 2018, and over all years is reported to provide a general indication of month and day of first flower over the study period (conditional on the estimated trend). Also included are the shift, in days per decade (with 95% highest posterior density intervals provided parenthetically) and chances of a non-zero trend.

Species	Expected onset of flowering date			Shift in days per decade	Chance that shift is occurring (%)
	In 1982	Over all years	In 2018		
<i>O. tesota</i>	5/14	5/9	5/3	−3.26 (−4.03, −2.47)	100.0
<i>P. microphylla</i>	4/25	4/22	4/18	−1.94 (−3.19, −0.44)	99.0
<i>V. constricta</i>	4/23	4/21	4/19	−1.05 (−2.72, 1.06)	83.5
<i>F. splendens</i>	3/8	3/2	2/24	−3.32 (−5.35, −0.79)	99.6
<i>A. deltoidea</i>	3/4	2/24	2/16	−4.39 (−7.74, −1.30)	100.0
<i>E. farinosa</i>	1/28	1/28	1/27	−0.54 (−6.12, 5.19)	58.1

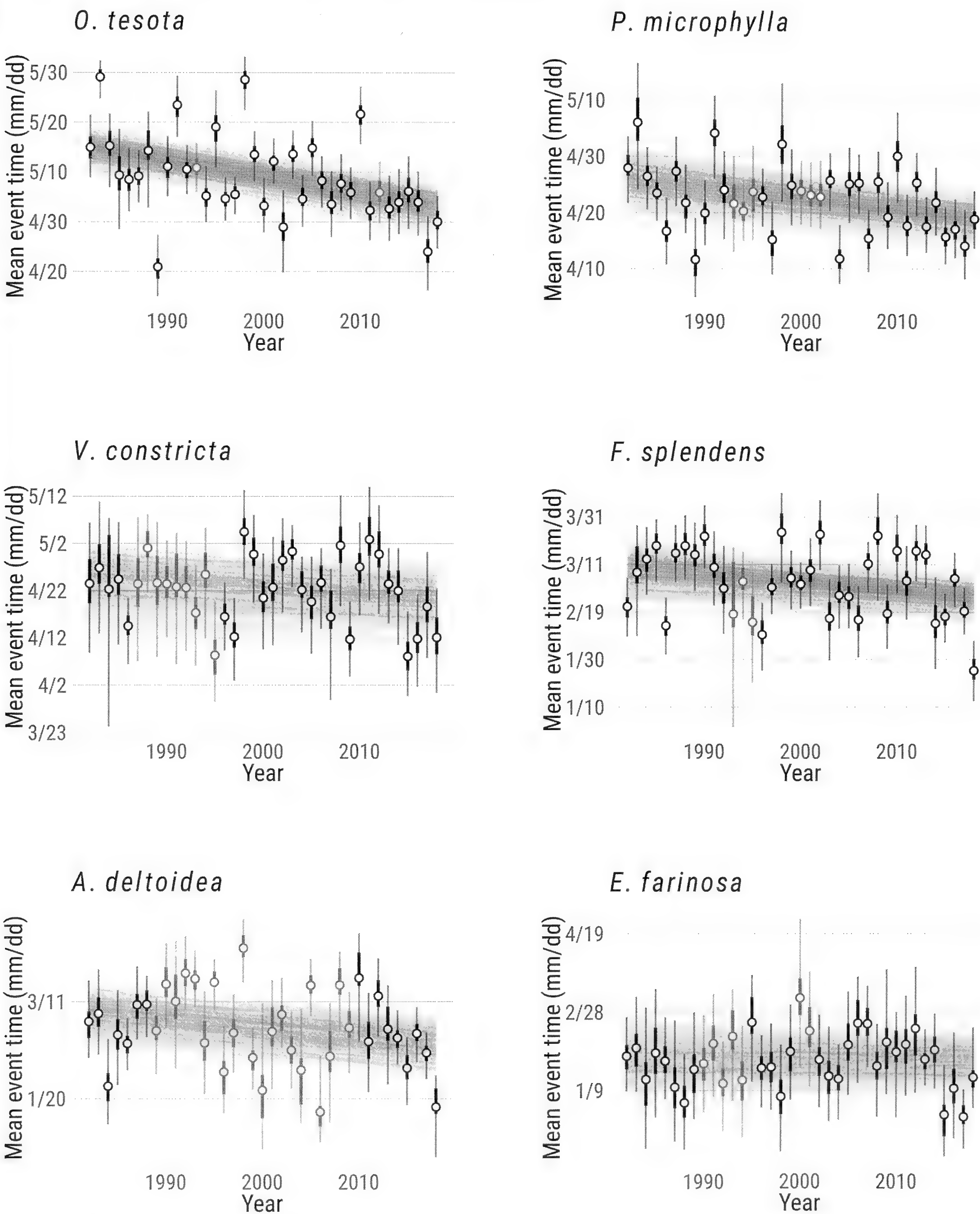


FIG. 3. Median (points), 50% highest posterior density interval (HDI; inner thick error bars), and 95% HDI (outer thin bars) of predicted mean flowering times (y-axis) by species by year (x-axis). Note that the y-axes differ for each species because the phenological window for cool-season flowering for each species is different. Black error bars correspond to years during which a given species was observed. Gray bars denote unsampled years. The semi-opaque gray lines beneath individual mean flowering times provide an estimate of trend – i.e., phenological advance (decreasing slopes) or delay (increasing slope). Trend results were developed using a truncated normal linear regression of mean flowering times on year at each iteration of the MCMC algorithm used to fit the model for each species, which appropriately propagates uncertainty from the phenological model into the estimates of trend. The spread in gray lines represents uncertainty in the trend. For more complete information on trend and associated uncertainty, see Table 2.

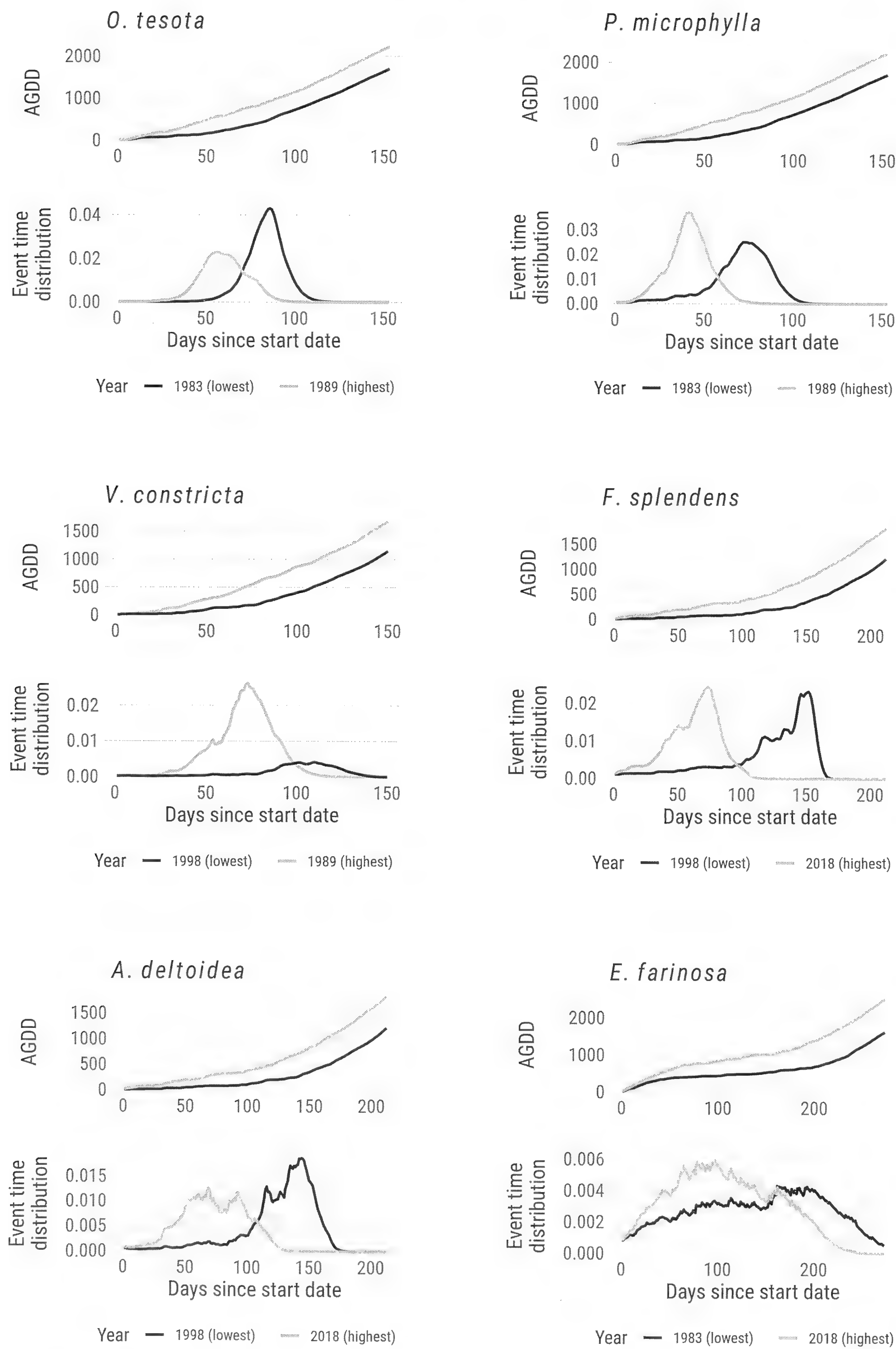


FIG. 4. The expected influence of accumulated growing degree days (AGDD) on the distribution of flowering times in the two years representing the extreme ends of AGDD for each species. The lowest (dark grey lines) and highest (light grey lines) AGDD years varied by species because the phenological window within which AGDD was summarized varied by species, and the years for which we had data also varied by species. These results are based on the medians of all coefficient values reported in Fig. S1. A with all other covariates held at their daily means. We show relative time to flowering (as opposed to date; mm/dd) within each species' phenological window along the x-axis so that effect size is more apparent. See Table 1 for detail on species' phenological windows. All species show an advancing trend (earlier flowering) with increasing AGDD, and the strength of this effect helps account for much of the trend seen in Fig. 3.

TABLE 3. THE EFFECT OF CHANGE IN THE WEATHER COVARIATES ON THE TIMING OF FLOWERING. We shifted each covariate by increasing its value each day by 30% of the mean across that day’s range of variation in the historical record. Here we report the +30% shift, but the –30% shift in these covariates would produce the opposite effect on timing of flowering (Fig. S2). Delays and advances are reported. Unmodeled effects are indicated as empty cells (“-”). See Figs. S1 and S2 for more detail on effect size.

Species	AGDD	VPD	2-week rainfall	Rainless days	Monsoon rainfall	# Freezes	PDO
<i>O. tesota</i>	advance	advance	delay	-	delay	delay	-
<i>P. microphylla</i>	advance	delay	advance	-	-	-	-
<i>V. constricta</i>	advance	delay	delay	-	-	-	-
<i>F. splendens</i>	advance	delay	advance	-	-	delay	-
<i>A. deltoidea</i>	advance	delay	delay	advance	advance	-	advance
<i>E. farinosa</i>	advance	advance	delay	delay	-	-	advance

VPD was important for every species, advancing flowering for *O. tesota* and *E. farinosa* and delaying the flowering for the other species (Fig. S2). Rainless days, antecedent monsoonal rainfall, the number of freezing events, and the PDO were only important for some species. Specifically, PDO advanced flowering in *A. deltoidea* and *E. farinosa*, and number of freezing events delayed flowering in *O. tesota* and *F. splendens* , while rainless days and antecedent monsoonal had mixed effects on flowering for the species they affected (*O. tesota*, *A. deltoidea*, and *E. farinosa*; Fig. S2). Divergent responses (advance vs. delay) across species to moisture-based climate variables was also apparent when we shifted the climate variables by $\pm 30\%$ each day relative to that day’s mean (Table 4, Fig. S2), and when we compared models representing the extreme ends of a particular climate variable while holding all other variables constant at their daily means (Fig. 5).

DISCUSSION

Our study sought to advance our understanding of phenological changes of keystone Sonoran Desert species and their response to climate variability nearly thirty years after Bowers and Dimmitt (1994) laid the foundation for dryland plant phenology. Using a new Bayesian model that sharpens our analysis of a unique long-term phenological data set, we demonstrate that the flowering phenology of keystone species in the Sonoran Desert are generally advancing and tied to rapidly changing temperatures – specifically more rapidly accumulating growing degrees early in the year. These results are consistent with others who have documented an advance in onset of flowering in woody species within the Sonoran Desert (Bowers 2007; Crimmins et al. 2010).

A data set collected by a single observer over a period of seventeen years immediately prior to the start of our phenological observations provides another opportunity to corroborate our results. McGinnies (1983) recorded the onset, peak and end of flowering for five of our six focal species (all but *A. deltoidea*) from 1966 through 1982 in a location 15 miles east of our study site. Comparing expected mean times for onset of flowering over our study period (1982–2018) with those reported by McGin-

nies for the seventeen years prior (1966–1982), we find that since the early 1980’s, the onset of flowering has advanced for every species by days to weeks (2–16 days in our dataset, with an average across all six species of ~ 9 days).

Flowering phenology of each species was also affected by short-term (2-week) windows of rainfall and VPD. However, unlike each species’ consistent responses to variation in higher AGDD (advance), responses to moisture-based variables diverged across species. For example, whereas an increase in VPD advances flowering in *O. tesota*, it delays flowering in *P. microphylla* (Fig. S2). Some of the divergent responses across species to moisture-based variables may be explained by variation in functional traits that render them more or less sensitive to changes in temperature and moisture. Despite differences in responses to moisture variables, years in which flowering was exceptionally early or late were consistent across these tree species. For example, for both *O. tesota* and *P. microphylla*, the latest onset of flowering was observed in 1983 (Fig. 3). This consistency is likely a result of a similar response to AGDD, which outweighs divergent responses to moisture-related variables.

In contrast to our findings of an approximately two-day advance per decade from 1982 through 2018 for *P. microphylla*, Crimmins et al. (2010) noted a delay in flowering from 1984 through 2003. These differences may reflect a difference in two populations of *P. microphylla*, with our study population in the drier Tucson Mountains, and the other population in the more mesic foothills of the nearby Santa Catalina Mountains. Advancing flowering in our drier site may help ensure water availability during cooler months when potential evapotranspiration is low. An alternative explanation is that the trend has changed direction with the rapid warming in the additional fifteen years since 2003.

In comparison to the tree species, *E. farinosa* and *A. deltoidea*, two small shrubs, showed greater sensitivity to other climate drivers, including PDO and rainless days, and also antecedent monsoonal rainfall for *A. deltoidea*. While these shrubs can effectively modulate their water-use efficiency (Driscoll et al. 2020), they may be more sensitive to changes in water availability than our other species.

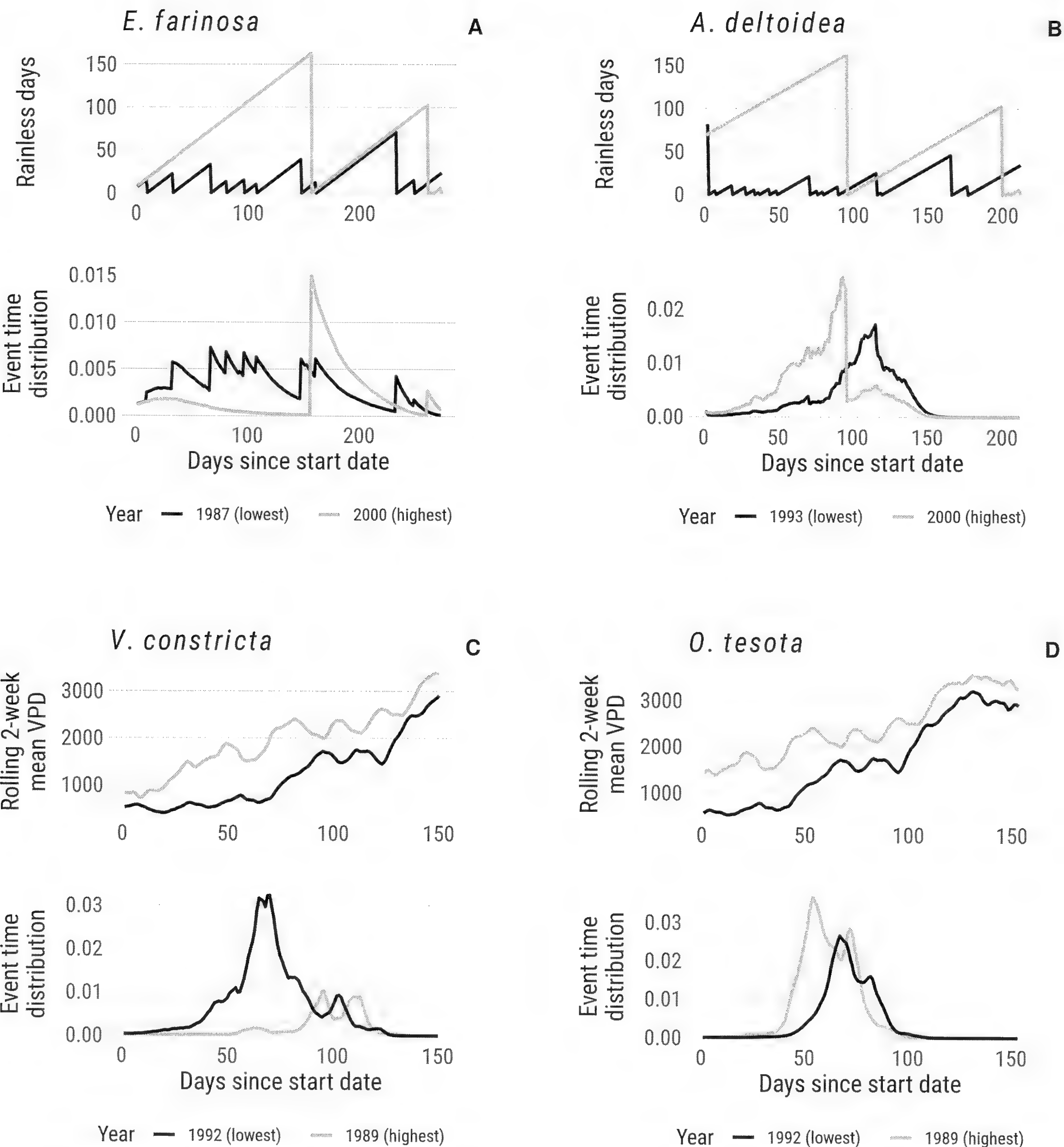


FIG. 5. Contrasting effects of moisture on flowering times across different species. Panels A–B illustrate the expected influence of rainless days on the distribution of flowering times in the two years representing the extreme ends of rainless days for each species, with all other covariates held at their daily means. Panels C–D show the expected influence of vapor pressure deficit (VPD) on the distribution of flowering times in the two years representing the extreme ends of VPD for each species, with all other covariates held at their daily means. These results are based on the medians of all coefficient values reported in Fig. S1. We show relative time to flowering (as opposed to date) along the x-axis here so that effect size is more apparent. See Table 1 for start dates.

Although AGDD pushes *E. farinosa* towards earlier flowering, any trend in the onset of flowering may be countered by its ability to respond to short-term rainfall (Fig. 5A). In contrast to the other five species, the probability of flowering in *E. farinosa* does not exhibit a distinct peak, but rather gradually rises and hovers around a near constant probability

for at least three months before gradually declining (Fig. 4).

Mode of pollination may also constrain flowering phenology. The probability of flowering in *E. farinosa* and *A. deltoidea* exhibits the opposite response to rainless days. In *A. deltoidea* the probability of flowering increases with increasing

number of rainless days, dropping precipitously after significant rainfall events (Fig. 5B), perhaps an adaptation for this wind-pollinated species. The opposite pattern is seen in *E. farinosa*, an insect-pollinated species. Its probability of flowering increases after each rainfall event (Fig. 5A). More generally, many of the dominant species in our study are sparsely distributed across landscapes in the Sonoran Desert and rely on pollinators, especially native bees (Simpson and Neff 1987). Future climate change could disrupt plant-pollinator interactions thereby affecting the reproductive output of these plant species.

The Sonoran Desert, like many global drylands, has warming rates much higher than other regions due to low vegetation cover and low soil moisture that enhance increases in temperature and aridity (Huang et al. 2016). Climate projections show a large amount of warming and drying expected for this site, with an increase of around 6 °C in mean annual temperature and more than 1 kPa in mean annual VPD, compared to the 1980s. Although the Sonoran Desert has experienced a reduction in mean annual precipitation since the 1980s (Munson et al. 2012), future projections show that precipitation may increase by 280 mm or decrease by as much as 120 mm. Regardless of the directional change in precipitation, increases in temperature and VPD alone will drive down soil moisture, thereby creating additional stress in plant water availability (Grossiord et al. 2020). Flowering phenology may continue to respond to climate trends moving forward, with higher divergence up to a limit, and pollinator activity and other non-climatic factors may interact to constrain future phenology.

With increasing temperature, we can generally expect an increase in accumulated growing degree days and advancements in flowering for these keystone desert species in the future. However, our models suggest that advancements in flowering may be accentuated or countered by concurrent changes in VPD, freezing events, and rainfall, depending on the species. Importantly, our uncertainty in the effect of any given covariate value on mean flowering time increases as covariate values become more extreme, which may be the case under future climates. When covariate values are more extreme (i.e., further from the overall mean covariate value in either direction) a relatively small amount of uncertainty in model parameters propagates into much larger uncertainty in outcomes. In other words, as covariate values become more extreme, there is less confidence in model predictions, and accordingly, variance is higher. This artifact (higher uncertainty and greater spread in possible mean flowering times) can be seen in our predictions for certain years that had extreme covariate values (Fig. 3).

Multi-decadal observational records and the increasing availability of powerful modeling techniques have improved our knowledge of how moisture availability affects flowering phenology, but our understanding remains incomplete. A focus

on VPD in flowering phenology is particularly new, but initial work shows that developing and maintaining flowers is water-costly, especially in desert regions, for two reasons. First, water use during flowering can be high if flowers transpire more water than leaves, including corollas with greater surface area than leaves (Lambrecht 2013; Berg et al. 2019). Second, water content in corollas can be higher in some flowers that have a greater pectin content to maintain flowers, a common pattern in arid regions (Teixido et al. 2019). Our results provide evidence of both advancing or delaying floral development in response to increasing VPD, which is a way to avoid flowering during a time with high moisture deficit. The relationship between atmospheric water demand and the timing of these events is likely complex, including a plant's change in water status and functional need to allocate water rather than a direct relationship with atmospheric demand (e.g., Reich and Borchert 1984). Regardless, including short-term rolling windows of both VPD and rainfall, in addition to temperature, improved our phenological models, suggesting moisture availability in all its various forms, from dry soils to a thirsty atmosphere, is relevant to phenological processes and demands greater mechanistic understanding. This issue is especially true now that atmospheric demand is continuing to increase under climate change.

Higher temperature and growing degree days are associated with earlier pollinator emergence and metabolic and physiological processes that induce flowering (Hegland et al. 2009). Although the physiological processes that govern development in both insects and plants are regulated by temperature, this does not ensure phenological shifts in response to increasing temperatures will be similar in size for insects and their host plants (Forrest and Thomson 2011). From a pollinator's perspective (e.g., a Monarch Butterfly), this can result in reduced food availability during reproduction or migrations. While we did not monitor pollinator activity in our study, subsequent studies can help reveal to what degree shared phenological trends are driven by attracting a similar suite of pollinators and whether or not advances in flowering will impact the probability of successful pollination. Species likely to be most affected by changes in pollinator availability are obligate outcrossers, including *Encelia farinosa* (Ehleringer and Clark 1988), though plant population reductions may not be immediately realized for other long-lived Sonoran Desert species.

We document a trend of advanced onset of flowering in five of our six focal species and divergent responses to moisture-based variables. Much work remains to understand the implications of this trend and other phenological changes (e.g., duration of flowering) for the fitness and demography of our focal species and the implications for the broader ecological community. Species that inhabit highly variable environments, such as the Sonoran Desert might be expected to exhibit greater adaptive

plasticity. Within just these six species, we observed a remarkable diversity of strategies to cope with limited and highly variable moisture availability. Such species may be more likely to survive in novel environmental conditions created by climate change.

CONCLUSIONS

Bowers and Dimmitt (1994) were among the first to quantitatively demonstrate changes in the phenology of Sonoran Desert woody species. Nearly three decades later, botanists at the Arizona-Sonora Desert Museum continue to collect phenological data with the same methodology on the same plant populations resulting in one of the longest records of arid plant species phenology in the world. We applied a new Bayesian model to an additional two decades of monitoring to take a more nuanced look at the phenology of these plant species. The new modeling approach we used is an improvement over simple correlative approaches and can advance future phenological studies by examining effects on daily probabilities of flowering, and by extension, the full distribution of flowering onset times. Importantly, improvements in modeling allow us to consider climate drivers at multiple temporal scales, from daily to antecedent. Future work could improve on this study by modeling intensity of response (the proportion of plants flowering) rather than simple presence / absence alone.

We provide evidence of a trend towards earlier flowering (1–4 days per decade) for five of the six species analyzed, likely linked to rising temperatures. Although dominant Sonoran Desert species similarly advanced in our study in response to greater AGDD, we found – like Bowers and Dimmitt (1994) – that measures of moisture availability were also important determinants of flowering phenology for some species that either countered or enhanced the advancing trends. The dominant species we evaluated showed either no effect or contrasting phenological responses to the same metrics of moisture availability, suggesting individualistic changes in phenology and the potential for divergent plant community flowering patterns under future climate change.

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DATA AVAILABILITY

Code and data for implementing the analyses described here has been deposited in a public, online repository (doi:

<https://doi.org/10.5281/zenodo.5245458>). All supplementary figures and tables can also be found in this repository.

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BERRIES IN WINTER: A NATURAL HISTORY OF FRUIT RETENTION IN FOUR SPECIES ACROSS ALASKA

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ABSTRACT

Plants with persistent fleshy fruits that last throughout fall and into winter and spring are an important source of nutrition for animals and people in boreal, subarctic, and arctic regions, but little information on fruit retention or loss is available for these regions. We evaluated fruit loss for four species across Alaska using data from our Winterberry community science network. Plants of *Rosa acicularis* Lindl., *Viburnum edule* (Michx.) Raf., *Vaccinium vitis-idaea* L., and *Empetrum nigrum* L. were monitored on a weekly basis throughout fall until snow cover and again after snow melt in 24 communities in six ecoregions in 2016–2020. Observers counted fruits and classified them into “unhealthy” (dried, rotten, or damaged) or “healthy”. Number of fruits lost per day (absolute loss rate) decreased over the course of the fall, but percent of fruits lost per day (relative loss rate) was constant for all species except *E. nigrum*, where it declined throughout the fall. Rates of loss were similar across ecoregions and climatic gradients, although for *V. vitis-idaea* the two most southern sites had the lowest relative loss rates and for *E. nigrum* the sites warmest in summer had the lowest loss rates. Fruit loss pulse events (>15% fruits lost in one week) were uncommon (<5% of weekly observations). At the time of persistent winter snow cover, plants retained 25–50% of fruits, with higher retention in more southern ecoregions. During winter, both relative fruit loss and absolute fruit loss rates dropped compared to fall, but in spring they rebounded to fall levels. Low proportions of unhealthy fruits in *E. nigrum* and *V. vitis-idaea* were in part due to rapid abscission of unhealthy fruits, while the other two species tended to retain unhealthy fruits. We estimate that vertebrate frugivores obtain 6–45 × as many fruits in fall as do decomposers / invertebrates. The higher loss rates during the snow-free seasons and constant rates of fruit loss for most of the focal species and locations suggest that longer falls and earlier fruit ripening will lead to lower fruit availability to animals in winter and spring.

Key Words: arctic, boreal, citizen science, *Empetrum nigrum*, frugivory, *Rosa acicularis*, *Vaccinium vitis-idaea*, *Viburnum edule*.

In temperate zones, most plants that use vertebrates as seed dispersers lose a high proportion of their fruits over a short time period following ripening (e.g., Thompson and Willson 1978; Stiles 1980; Sargent 1990), while a smaller number of species retain their fruits throughout the fall and winter (e.g., Stiles 1980; Borowicz and Stephenson 1985; Jones and Wheelwright 1987; Sallabanks 1992; Gervais and Wheelwright 1994). In boreal, subarctic and arctic regions, plants with persistent fruits are well represented in the woody shrub flora: they include species in the Ericaceae (e.g., *Vaccinium vitis-idaea* L., *V. oxycoccos* L., *Arctostaphylos uva-ursi* (L.) Spreng.), Empetraceae (*Empetrum nigrum* L.), Caprifoliaceae (*Viburnum edule* (Michx.) Raf.), and Rosaceae (e.g., *Rosa acicularis* Lindl.) (West 1982; Pullainen and Tunkkari 1991; Aiken et al. 2007; Krebs et al. 2010; Hupp et al. 2013; Mulder unpublished data). A few herbaceous species also retain their fruits for extended periods of time (e.g., *Cornus canadensis* L. (Cornaceae; West 1982), *Actaea*

rubra Bigelow (Ranunculaceae); B. Spellman, Natural Resources Conservation Service Alaska, personal communication), and Convallariaceae (e.g., *Maianthemum racemosum* Link.; M. Goff, personal communication). Persistent fleshy fruits constitute an important component of the late fall, winter, and early spring diet for many animals at times when other food is scarce, including microtine rodents (e.g., northern red-backed voles, *Myodes rutilus*, West 1982; Krebs et al. 2010), foxes (e.g., Dell’Arte et al. 2007; Needham et al. 2014), bears (McLellan and Hovey 1995; Munro et al. 2006), migrating waterfowl (Hupp et al. 2013), and birds that overwinter in the north like ptarmigan and grouse (Pullainen and Tunkkari 1991; Wegge and Kastdalen 2008). Fruits may also be an important source of water in springtime to birds such as grouse and geese (Pullainen and Tunkkari 1991; Hupp et al. 2013). Fleshy fruits are of high nutritional and cultural importance to Indigenous and rural communities throughout Alaska and Canada (Kari 1987; Bellew et

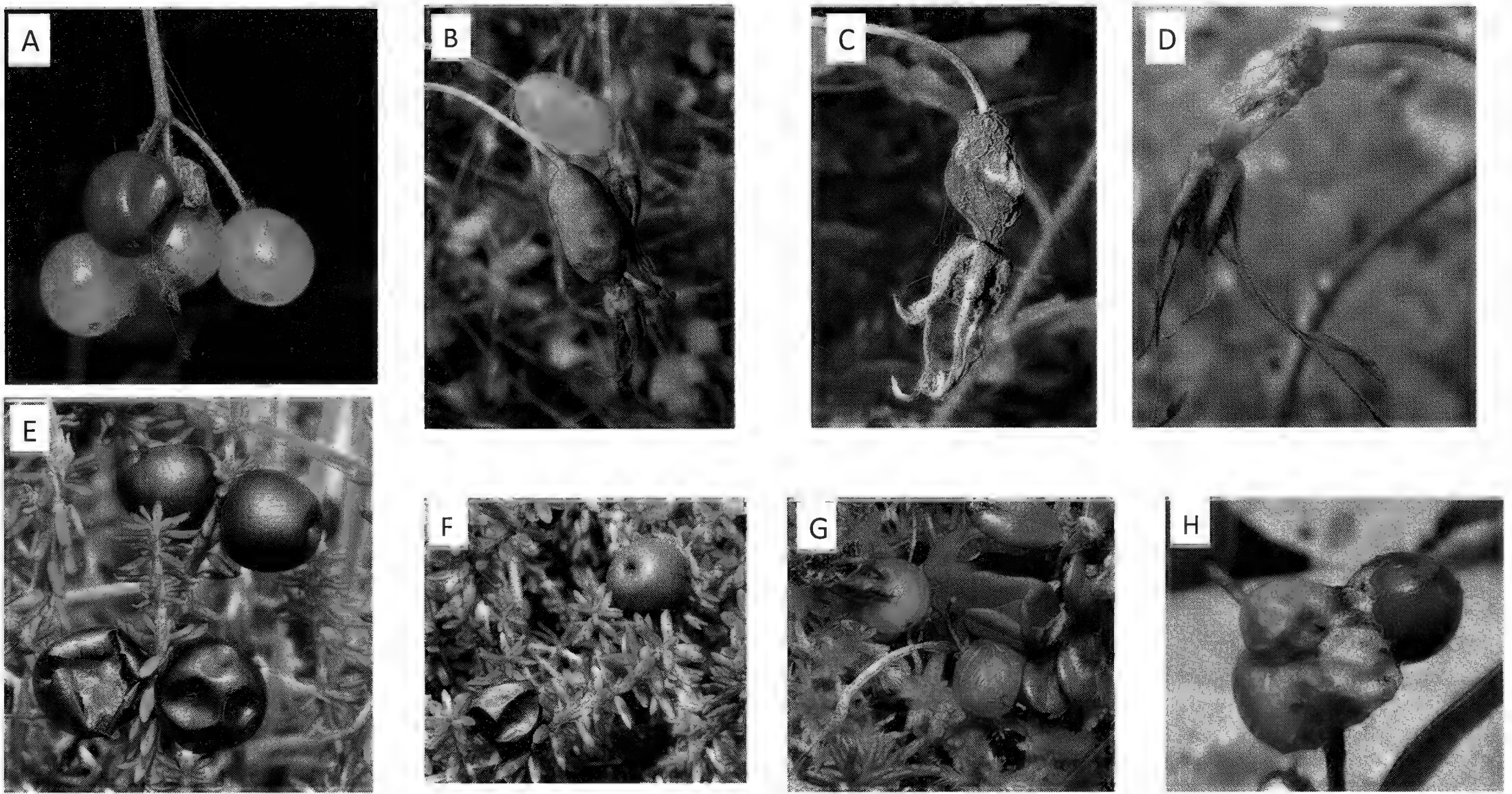


FIG. 1. States of fruits of the four focal species. A. Fruits of *Viburnum edule* (Highbush Cranberry) in ripe (bottom 3), infected (top left) and shriveled (top center) stage. B–D. Fruits of *Rosa acicularis* (Prickly Rose) in ripe and infected (B), dry (C) and damaged (D) state. E–F. Fruits of *Empetrum nigrum* (Crowberry) in ripe (top 2 in E and top in F), damaged (bottom left in E) and shriveled (bottom right in E, bottom in F) stages. G–H. Fruits of *Vaccinium vitis-idaea* (Lingonberry) in ripe (top in both), shriveled (bottom in G) and infected (left 3 in H) state. All images courtesy of A. Ruggles, except for D (C. Mulder).

al. 2006; Hupp et al. 2015), and people often collect species like *Vaccinium vitis-idaea* and *V. oxycoccos* in springtime (Kari 1987; Aiken et al. 2007), as reflected in the Denai'na name for *V. vitis-idaea*: “Hey Gek’a” or “Winter Berry” (Kari 1987).

A common explanation for why some species retain their fruits is that competition with other species for seed dispersers is reduced during the colder months (Stiles 1980; Jones and Wheelwright 1987). However, plants with persistent fruits also face a challenge: fruit retention over many months may result in high damage by microbes and invertebrates (Thompson and Willson 1978; Herrera 1982). To counter this, plants invest in compounds, such as the organic acids found in *Vaccinium* species (Cipollini and Stiles 1992; Aiken et al. 2007; Ermis et al. 2015; Stobnicka and Gniewosz 2017), *Arctostaphylos uva-ursi* (L.) Spreng. (Cipollini and Stiles 1992), and *Viburnum opulus* L. (Jones and Wheelwright 1987); such compounds may make fruits less palatable to vertebrate seed dispersers, resulting in slower dispersal overall (Stiles 1980; Jones and Wheelwright 1987; Cipollini and Stiles 1993). Some species may also contain non-pathogenic microbes that reduce the growth of pathogenic microbes (Cipollini and Stiles 1993). At high latitudes ($>50^{\circ}\text{N}$), several factors may further select for persistent fruits. First, the role of ground thaw in triggering flower development combined with the short growth season results in highly synchronous flowering and ripening of fruits across species in early fall (Barr et al. 2009;

Wolkovich and Cleland 2011; Mulder and Spellman 2019). This likely exacerbates competition for seed dispersers in early fall but results in fewer choices for frugivores later in the season. Second, fruit loss to microbes during extended periods on the plant may be lower than at more southern latitudes because of cool fall temperatures and, in areas with a strongly continental climate, low humidity.

Despite the demonstrated importance of fruit retention to animal populations and the potential importance to the plants themselves, very few studies have directly measured fruit retention or loss through fall and winter in high latitude wild plants. Numerous studies have attempted to indirectly evaluate the abundance of overwintering fruits in the guts, crops, and fecal matter of animals (see examples above). This information, however, does not provide an accurate measure of fruit removal over time, as it does not take into account changes in consumer population size or behavior, or availability of other foods. Furthermore, these data do not provide clear insights into the costs and benefits for plants of retaining fruits, such as how seed dispersal is distributed over time, and when and to what extent fruits are lost to decomposers. Although in some species a high proportion of fruits remain in a “healthy” state, others appear infected or shriveled, even while retained on the plant (Fig. 1). Thus, a portion of the carbon and nutrients in the fruits are likely obtained by decomposers rather than frugivores even before the fruits are lost from the plant,

and a shift in the proportion of fruits in different states (e.g., due to changes in environmental conditions) would affect food web structure. The one study we were able to find in a high latitude ecosystem that addressed losses to consumers vs. decomposers documented the fate of *Cornus canadensis* fruits from peak crop to snowfall over 3 yr and found that the majority of the berries each year were removed or damaged by consumers, while decomposers infected an average of 18% of the remaining fruits (Burger 1987). The study pointed to a need for further documentation of the fate of berries in the fall and winter season within the high latitudes, where the abundance and condition of overwintering fruits may play a heightened role in winter food webs relative to other systems where other foods are more available.

Historical datasets and surveys of longtime berry pickers suggest timing of berry production is becoming more variable (Hupp et al. 2015; Spellman and Mulder 2016). Year to year variation in the timing of fruit loss and condition of the fruit is likely influenced by long term increases in high-latitude temperatures (Wolken et al. 2011) and growing season length (Mulder and Spellman 2019). Decreased precipitation as snow fall may result in an even earlier start to the growing season (Littell et al. 2018), further lengthening the growing season. In the far North, the timing of berry ripening is driven primarily by the timing of flowering, which in turn is driven by spring conditions (time of snow melt and temperature) (Mulder and Spellman 2019). As a result, berries may be exposed to consumption during late summer and early fall (above-freezing conditions) for a longer period. Will this result in a greater loss of fruit in late summer and fall, leaving fewer resources for consumers in late fall, winter, and spring? Will it result in greater carbon and nutrient flow to decomposers? We need a basic understanding of the natural history of fruit retention to start to answer these questions.

In this study, we tracked fruit retention in four plant species with very wide distributions across northern North America. We developed a youth-centered, state-wide community science network called “Winterberry” to collect direct observations of fruit retention from the time of ripening until snow cover, and again from snow melt into the spring. Our data span 46 sites in 24 communities across 6 ecoregions of Alaska and were collected during a 4-yr period (2016–2020). We asked the following questions for each species at the site level:

1. How does rate of fruit loss (number of fruits and percent of fruits) and the proportion of fruits in a “healthy” state (defined as fruits that are not rotted or shriveled and have no obvious invertebrate damage) differ between seasons (fall, winter, and spring)? We predicted lower absolute loss rates in winter than in fall or spring due to lower animal and microbial activity. Rates of fruit loss in tall (above-snow) or short (below-snow) species may depend on the relative importance as frugivores of birds

and above-snow mammals, such as foxes, compared to subnivean animals such as microtine rodents.

2. How do fruit loss rates (absolute and relative) and proportion of healthy fruits change over the course of the fall? These are the result of opposing effects of number of frugivores and competition for frugivores, both of which are expected to be greater in early fall, as well as by loss rates due to abscission (expected to be greater for unhealthy fruits).
3. Do fruit loss rates and the proportions healthy fruits differ between ecoregions of Alaska? We do not have *a priori* predictions for rates of removal, as they will depend on both the total availability of frugivores and competition with other species for seed dispersal. We expected greater proportions of healthy (non-diseased) fruits in dry ecoregions, such as the Intermontane Boreal Zone, and higher rates of loss in wetter locations, such as the Coastal Rainforest and Aleutian Meadows ecoregions (Fig. 2).
4. What proportion of fruits transition from healthy to unhealthy (rotten, shriveled, or damaged by invertebrates), and at what rates are healthy and unhealthy fruits lost from the plant? Does this differ by season or by ecoregion? High losses of healthy fruits suggest a large role of vertebrate frugivores compared to decomposers and invertebrate frugivores, while high rates of transition from healthy to infected fruits suggest decomposers play a dominant role in fruit loss.

We asked an additional set of questions at the individual plant level: Do more fruits on a plant affect: a) the probability of at least one fruit being lost through either removal or abscission, or b) the proportion of fruits lost?

METHODS

Four focal species were selected: *Rosa acicularis* (Prickly Rose), *Viburnum edule* (Highbush Cranberry), *Vaccinium vitis-idaea* (Lowbush Cranberry or Lingonberry), and *Empetrum nigrum* (Crowberry). Species selection was based on the following traits: 1) a wide distribution across Alaska (Fig. 2A) and high latitudes in North America or Eurasia (Hultén 1968); 2) retention of fruits throughout fall and winter, 3) high local abundance in the communities involved in our community science program, and 4) high importance to people in Alaska and the circumpolar North (Hupp et al. 2015). *Rosa acicularis* and *V. edule* are generally >0.5 m tall in most locations and are therefore only partially covered by snow during winter, while *V. vitis-idaea* and *E. nigrum* are < 0.3 m tall and are completely covered by snow during most of the winter in most locations.

Plants were monitored at 46 sites in 24 communities by \approx 1500 volunteers. All volunteers participated with free, informed, and prior consent under our University of Alaska Fairbanks IRB plan submitted and approved for our program (UAF IRB #1062412-5), which also included human subjects data for our education research (for more information on the education research see Spellman et al. 2019).

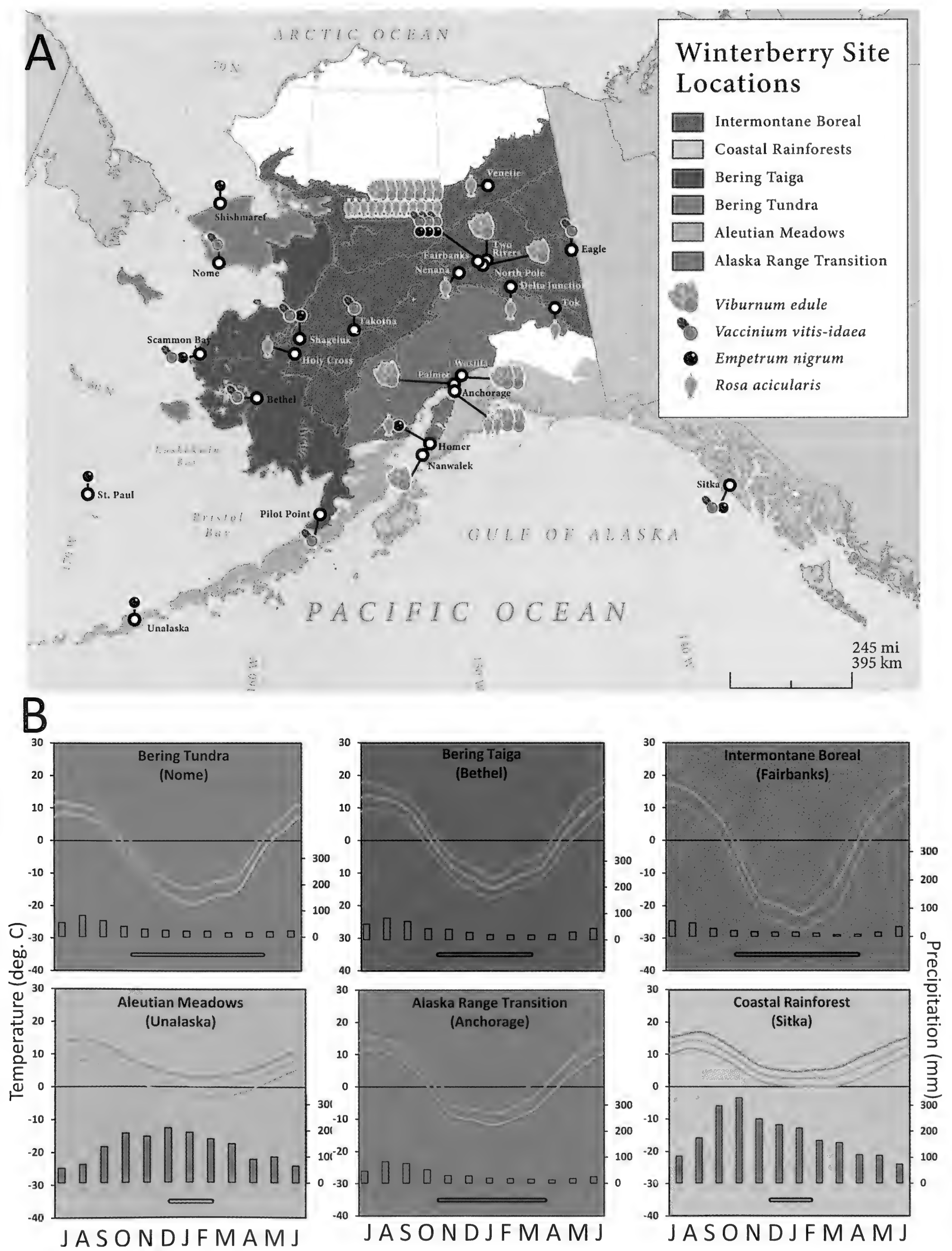


FIG. 2. Ecoregions and their climates. A. Collection site locations across the six ecoregions based on Nowacki et al. (2001). The number of symbols corresponds to the number of sites at that location. B. Temperature and precipitation profiles for a representative town in each ecoregion based on 1981–2010 means (U.S. Climate Data, 2020). Note that profiles are centered on mid-winter. The bar across the bottom of each graph represents time during which the ground is covered in snow, for most regions defined as from >50 mm of snowfall after the minimum daily temperature < 0°C until the mean daily temperature > 0°C. In the Coastal Rainforest and Aleutian Meadows habitats there is no season-long snow cover, but most snow fall occurs in December, January, and February.

Individuals, families, youth groups and educators tracked the abundance and condition of the fruits on a minimum of 20 marked individual plants with a minimum of 100 fruits across the plants. Individual plants were added and marked as needed to meet the minimum 100 fruits for the start of the monitoring season. Each individual plant was observed each week at all of the sites, and the total number of fruits remaining on the plant was recorded in five condition categories: “unripe” (defined as having green color still visible on the fruit), “ripe” (fully red or black in color), “rotten” (discolored and squishy or moldy), “dried” (berry is dehydrated, shriveled and hard to the touch), and “damaged” (fruit skin is ripped or has holes in it) (Fig. 1). Since observers reported having difficulty distinguishing between rotten and dried, especially later in the season for *R. acicularis*, after the first year we added a “rotten or dried” category. In some instances, volunteers tracked multiple species within their site, each with a minimum of 20 individual plants. All volunteers received training setting up their site and in the classification of these berry conditions. Weekly monitoring began as soon as the fruit began to ripen or at the start of the school year for the youth groups in mid-August, ceased when the snow fell and remained at the site so as not to disturb the subnivean environment, and resumed in the spring when the snow had melted until the berries had all been removed, or the first flowers appeared. Observers were encouraged to report sightings of animals or animal sign. Data quality was assured through a rigorous quality review process that included consultation with each group of volunteers in a “data jam” session. Mean data quality issue rates were very low (only 2.7% of all observations). The full protocol for citizen scientists is available in Appendix S1. Total number of plant observations were 3559 for *R. acicularis*, 3676 for *V. edule*, 2754 for *V. vitis-idaea*, and 1507 for *E. nigrum* (grand total = 11,496 observations).

Sites spanned six of the eight “unified ecoregions” of Alaska: Bering Tundra, Bering Taiga, Intermontane Boreal, Alaska Range Transition, Coastal Rainforest, and Aleutian Meadows ecoregions (Nowacki et al. 2001) (Fig. 2, Table 1; see Appendix 1 for details of data collection). These ecoregions are described according to broad similarities in climate, vegetation and disturbance regime, and represent polar, boreal, and maritime-like systems. Annual temperature and precipitation means for representative locations within each ecoregion are shown in Figure 2B. Bering Tundra sites are characterized by a mix of maritime and polar climates, with sea ice and dry winds in winter, and cool, moist conditions after spring break up. Soils are underlain by continuous permafrost, and vegetation is treeless tundra. Bering Taiga has a moist polar climate, with shrub tundra and wetlands dominating the discontinuous permafrost landscape. Intermontane Boreal sites are characterized by a strong continental climate with

very cold winters and warm summers. The permafrost is discontinuous and vegetation is dominated by White Spruce (*Picea glauca* (Moench) Voss, Pinaceae), Birch (*Betula neoalaskana* Sarg., Betulaceae), and Aspen (*Populus tremuloides* Michx., Salicaceae) trees on south facing slopes, and Black Spruce (*Picea mariana* Britton, Sterns & Poggenb., Pinaceae) and scrub tussock on north facing slopes and valley bottoms. Alaska Range Transition is a mix of maritime and continental climates, with an abundance of precipitation; soils are generally free of permafrost. Coastal Rainforest has a cool, hyper-maritime climate with only minor seasonal variation and long periods of cloudy, rainy weather. Permafrost is absent, and vegetation is dominated by rainforests of Sitka Spruce (*Picea sitchensis* (Bong) Carrière, Pinaceae) and Hemlock (*Tsuga heterophylla* Sarg., Pinaceae). Aleutian Meadows have a cool maritime climate, with cold ocean winds and persistent clouds and fog; the soil is permafrost-free and vegetation is dominated by low shrubs and ericaceous heath and grass. The town of Shageluk is in the transition zone between Intermontane Boreal and Bering Taiga and was assigned to Bering Taiga based on the greater similarity to sites in this ecoregion. If the sample size for an ecoregion was very low for a given species (one or two site-year combinations) and the location was on the edge of an ecoregion, it was combined with the most similar ecoregion for that species only; this occurred once for each species (Appendix 1).

Because our ecoregions are very large and some sites are near the border with another ecoregion, analyzing by ecoregion might miss changes across space driven by continuous variables such as temperature and precipitation. To classify communities by climate gradients, we obtained long term means (1961–1990) for temperature (mean daily averages for January, April, July and October) and precipitation (total precipitation as rain and as snow, number of months with only rain, only snow, or mixed) for each community (see Appendix 2 for details). Since we were interested in spatial variation rather than the effect of individual climate variables, we combined these nine variables in a principal components analyses (function *prcomp* in R version 3.5.2, R Foundation for Statistical Computing, Vienna, Austria) to generate climate axes. The first two axes explained 81% of variation (Appendix 2). The first axis, PC1, represented fall and winter conditions: in communities with high values winter came earlier (lower mean temperature in October), was colder (lower mean temperature in January), and lasted longer (more months of snow only) than in communities with lower values. The second axis, PC2, represented spring and summer conditions: in communities with high values the growth season started late (lower mean temperature in April) and was cool (lower July temperature) and there were more months of rain only than in communities with lower values (Appendix 2). We will refer to sites with

TABLE 1. SITE LOCATIONS AND DESCRIPTIONS. S = School. Species abbreviations: Empnig = *Empetrum nigrum*, Rosaci = *Rosa acicularis*, Vacvit = *Vaccinium vitis-idaea*, Vibedu = *Viburnum edule*.

Eco-region	Site name	Nearest town	Lat. (°N)	Long. (°W)	Elev. (m)	Habitat type	Species
Bering Tundra	Shismaref S.	Shishmaref	66.257	166.072	5	grasses, forbs	Empnig
	Anvil City Science Academy	Nome	64.541	165.411	30	tundra	Vacvit
	Kamenista	St. Paul	57.160	170.270	10	tundra	Empnig
Bering Taiga	Innoko River S.	Shageluk	62.654	159.532	42	boreal forest / tundra	Empnig
							Vacvit
	Holy Cross Elementary	Holy Cross	62.202	159.766	23	grasses, forbs	Rosaci
Inter-montane Boreal	Scammon Bay S.	Scammon Bay	61.842	165.582	5	tundra	Empnig
							Vacvit
	Bethel Regional High S.	Bethel	60.803	161.767	7	tundra	Vacvit
	Pilot Point Secondary Classes	Pilot Point	57.59	157.59	8	tundra	Vacvit
	John Fredson S.	Venetie	67.016	146.412	175	coniferous forest	Rosaci
	Denali Elementary School	Fairbanks	64.839	147.753	134	deciduous forest	Rosaci
	3rd and 5th grades						
	Tanana Middle S.	Fairbanks	64.846	147.665	138	coniferous forest	Rosaci
							Vibedu
	Randy Smith Middle S.	Fairbanks	64.857	147.754	134	deciduous forest	Vibedu
	Parkinson Yard	Fairbanks	64.862	147.918	200	coniferous forest	Empnig
							Vacvit
							Rosaci
	Parkinson #2	Fairbanks	64.803	147.997	277	mixed boreal forest	Vibedu
							Rosaci
	Smith Lake	Fairbanks	64.864	147.864	165	mixed boreal forest	Empnig
							Vacvit
							Vibedu
	Murie Trail	Fairbanks	64.860	147.845	180	Deciduous forest	Empnig
							Vacvit
	UAF satellite dish	Fairbanks	64.859	147.856	180	Deciduous forest	Rosaci
							Vibedu
	Mulder Yard	Fairbanks	64.896	147.813	227	Deciduous forest	Rosaci
							Vibedu
Alaska Range Transition	Weller Elementary	Fairbanks	64.887	147.592	281	Deciduous forest	Vibedu
	Two Rivers Elementary	Two Rivers	64.877	147.039	228	Deciduous forest	Vibedu
	Anne Wien Elementary	Fairbanks	64.858	147.746	133	Deciduous forest	Rosaci
	Hunter Afterschool Club	Fairbanks	64.833	147.730	135	Deciduous forest	Rosaci
	Watershed Elementary - KG	Fairbanks	64.827	147.868	134	Tall shrubs	Rosaci
	Watershed Elementary - Powerline Trail	Fairbanks	64.823	147.874	135	Tall shrubs	Rosaci
	Watershed Sit Spots	Fairbanks	64.820	147.878	136	Deciduous forest	Rosaci
	Boreal Sun S.	Fairbanks	64.825	147.735	134	Deciduous forest	Rosaci
	Arctic Light Elementary	North Pole	64.826	147.692	138	Tall shrubs	Rosaci
	North Pole Middle S.	North Pole	64.746	147.342	153	Coniferous forest	Vibedu
	Eagle Community S.	Eagle	64.787	141.205	272	Deciduous forest	Vacvit
	Nenana S.	Nenana	64.564	149.080	110	Tall shrubs	Rosaci
	Delta Future Farmers of America	Delta Junction	64.03	145.698	364	Deciduous forest	Rosaci
	Tok School	Tok	63.326	142.98	502	Deciduous forest	Rosaci
	Takotna Community S.	Takotna	62.989	156.043	121	Moss / lichen	Vacvit
	Mat-Su Career & Technical High	Wasilla	61.607	149.363	138	Deciduous forest	Vibedu
	Palmer Girl Scout Troop 849	Palmer	61.579	149.291	64	Deciduous forest	Vibedu
	Anchorage Botanical Garden	Anchorage	61.334	149.751	175	Mixed boreal forest	Vibedu
	Birch Hill	Anchorage	61.250	149.702	92	Mixed boreal forest	Vibedu
	East High Environmental Club	Anchorage	61.200	149.804	53	Deciduous forest	Vibdu
	Campbell Creek Bridge	Anchorage	61.165	149.768	84	Deciduous forest	Rosaci
	Polaris K–12 S.	Anchorage	61.163	149.853	40	Deciduous forest	Rosaci
	Wynn Nature Center	Homer	59.686	151.481	404	muskeg	Empnig
	Center for Alaska Coastal Studies HQ	Homer	59.646	151.524	28	herbaceous	Rosaci
Coastal Rainforest	Nanwalek S.	Nanwalek	59.354	151.921	11	Tall shrub	Vibedu
	Sitka Raptor Center	Sitka	57.052	135.314	16	muskeg	Empnig
Aleutian Meadows							Vacvit
	Unalaska City High S.	Unalaska	53.874	166.520	27	Shrub tundra	Empnig
	Eagles View Elementary	Unalaska	53.870	166.520	23	Shrub tundra	Empnig

high PC1 values as “winter cold” and ones with high PC2 values as “summer cold”. When plotted by PC1 and PC2, most communities clustered by ecoregion, but there was a cluster of five winter warm / summer cold communities that included one community from every ecoregion except Intermontane Boreal (Appendix 2); we therefore analyzed data both by ecoregion and by climate variables (PC axes).

Data Analysis

Because of large differences in data collection efforts in fall, winter, and spring, data were analyzed by season. “Fall” was defined as the period prior to season-long snow on the ground; data were usually collected weekly during this time period. “Winter” was the period when the ground and / or plants were covered with snow or ice; data were not collected during this period because of the potential for disturbance of the vegetation. “Spring” was defined as the period from re-initiation of data collection once the snow had melted until data collection ceased (either because the group disbanded or because the plants came into flower); at many sites data were collected only once or a few times in spring. These were good operational definitions for Bering Tundra, Bering Taiga, Intermontane Boreal, and Alaska Range Transition ecoregions, where snow melt events in winter are rare and short lived. In the two southern-most ecoregions, Coastal Rainforest and Aleutian Meadows, there was no season-long snow cover and data were collected continuously (though less frequently in winter). However, these ecoregions did have a 3-mo period (Dec–Feb) during which snowfall was considerable, so we defined this as “winter”.

We expected a lack of independence for plants within a site because a given consumer or decomposer could affect multiple plants, and for some species (e.g., *V. vitis-idaea*) multiple ramets might constitute a single genet. Therefore, we used the means per site for a given date for all analyses except those at the plant-level (the effect of number of fruits on probability of fruit loss), which used individual plants as the experimental unit.

Ideally, data collection would have started as most fruits were ripening, all plants would have been monitored weekly until snowfall, and weekly once the snow melted until no fruit remained. However, at many sites observations were not initiated until after all fruits had ripened, leaving the initial size of the cohort unknown. Many groups only recorded data once in the springtime (because the school year was ending or because very few fruits remained) and some groups recorded only in the fall (e.g., one-semester college or high school courses). As a result, the dataset for fall is much more extensive than for winter or spring. We therefore perform simple comparisons of patterns of fruit loss for the three seasons, followed by in-depth analyses of changes over the course of the fall season.

Comparisons between seasons. We first calculated the percentage of fruit lost in each season based on the change in fruit number at each site from start to end of the season. The number of year-site combinations decreased from fall to winter to spring; sites for which no data were recorded were excluded from the following season unless it was known that no fruits remained at the start of that season, in which case it was recorded as zero. We then expressed the change from the start to the end of the season as number of fruits lost per plant per day (including zeroes for sites where no fruits were present at the start of the season) and percent of fruits lost per plant per day (excluding sites where no fruits were present at the start of the season). Absolute rate of fruit loss is indicative of supply rates to animals, while relative rate of fruit loss, the complement of retention rate, represents the risk of loss from the plant for an individual fruit.

A third variable, proportion of fruits in a “healthy” state, was calculated as: $\text{prophealthy} = (\# \text{ unripe fruits} + \# \text{ ripe fruits}) / \text{total number of fruits}$. Unripe or ripe fruits were considered healthy while shriveled (dry), infected (rotten), or damaged fruits were considered unhealthy. We combined these three “unhealthy” categories because of difficulties distinguishing between the first two, and because damaged fruits, which were uncommon (<3.2% of all observations except for *R. acicularis* [8.3% of observations]), were usually also shriveled or infected.

For each of the three response variables (absolute and relative fruit loss and proportion of healthy fruits) we ran maximum-likelihood based mixed models with season as the fixed variables and the year-site combination as a random variable using the *lmer* function in the *lme4* package in R. Only sites for which data for both seasons being compared were available were included in these analyses. The number and percent of fruits lost per day were \log_{10} -transformed and prophealthy was arcsine-square root transformed to improve adherence to model assumptions. We controlled the family-wise error rate by comparing the P-values from the set of 36 tests to values generated using a Benjamini-Hochberg procedure (Benjamini and Hochberg 1995).

Changes over the course of the fall season. Intervals between monitoring were not consistent, and not every plant was monitored on every occasion. We therefore focused our analyses on changes between consecutive observations, rather than comparisons to the initial cohort. The absolute rate of fruit loss was calculated as:

$$\text{numlost} = \frac{(\text{fruits}_{t-1} - \text{fruits}_t)}{\text{days}}$$

Where fruits_{t-1} is the number of fruits at the previous observation, fruits_t is the number of fruits at the current observation, and days is the number of days

between the two observations. Similarly, the relative rate of fruit loss was calculated as:

$$perlost = \frac{(fruits_{t-1} - fruits_t) / fruits_{t-1}}{days} * 100$$

We evaluated changes in the absolute and relative loss rates as well as in proportion healthy fruits (prophealthy) over the course of the fall season, and tested for differences between ecoregions in these rates, restricting the dataset to the period for which data were available for at least 2 ecoregions. As for the season comparisons, numlost and perlost were \log_{10} -transformed and prophealthy was arcsine-square root transformed to improve adherence to model assumptions. We ran maximum-likelihood based mixed models that included Julian date and ecoregion as fixed variables and year and site as random variables using the *lmer* function in the *lme4* package in R. We started with the full model (including Julian date, ecoregion, and their interaction) and evaluated the impact of each term by dropping it from the model and comparing the simplified model to the more complex one using a chi-square value from a likelihood ratio test. If the variable removed explained a significant amount of the variation it was replaced before the next variable was dropped. We again controlled the family-wide error rate using a Benjamini-Hochberg procedure (Benjamini and Hochberg 1995).

To evaluate whether climate variables measured on a continuous basis explained variation not captured by the ecoregion classifications, we used the same approach to test for effects of PC1 (winter conditions) and PC2 (summer conditions) on numlost, perlost, and prophealthy. The full model included Julian date, PC1, PC2, and all 2-way interactions (plus year and site as random variables).

Transitions between states. We counted the number of fruits (healthy, unhealthy, or lost) on each plant during each observation period to determine whether healthy or unhealthy fruits are more likely to be lost by plants and whether this differs by season or ecoregion. There were three possible transitions: healthy to unhealthy, healthy to lost, and unhealthy to lost (we assumed that unhealthy fruits could not revert to healthy). Because we did not track individual fruits within plants, we were not always able to unambiguously determine the fate of each fruit. Ambiguity arose when a plant started with both healthy and unhealthy fruits and ended with fewer healthy fruits and at least some unhealthy fruits. For example, a plant with five healthy fruits and two unhealthy fruits (seven total) at the time of the first observation and with two healthy fruits and three unhealthy fruits (five total) during the next observation may have A) lost two healthy fruits and had one transition from healthy to unhealthy, or it may have B) lost two unhealthy fruits and had two transitions from healthy to unhealthy, or it may have C) lost one healthy and

one unhealthy fruit and had no other transitions. We calculated the proportion of fruits in each transition under two extreme scenarios. In Scenario 1, ambiguous losses are attributed to healthy fruits; this is expected if most losses are due to consumers and consumers are more likely to remove healthy fruits than unhealthy fruits. In the example above, this is option A. In Scenario 2, ambiguous losses are attributed to unhealthy fruits; this is expected if unhealthy fruits are more likely to be abscised than healthy fruits. In the example above this is option B. These two extreme scenarios bracket the range of possibilities for each of the three transitions. For example, the proportion transitioning from healthy to lost in option C ($1/5 = 0.2$) is intermediate between that of option A ($2/5 = 0.4$) and option B ($0/5 = 0$). Because we were comparing ranges rather than single values and because we do not know which of the two extreme scenarios is closer to reality for each species, we were not able to conduct statistical tests comparing ecoregions or seasons.

We estimated the proportion of healthy fruits lost per week to decomposers in fall and spring as:

$$decomposer_loss = \frac{propfruits_{HtoU} * propfruits_{UtoL}}{2}$$

Where $propfruits_{HtoU}$ is the proportion of fruits that transitioned from healthy to unhealthy and $propfruits_{UtoL}$ is the proportion of fruits that transitioned from unhealthy to lost.

In other words, we assumed this was a two-step process: first fruits partially decomposed (healthy to unhealthy), and then they dropped from the plant (we divided by 2 to produce a weekly rather than biweekly estimate).

We also calculated an index of the relative importance of vertebrate frugivory as:

$$propfruits_{HtoL} / decomposer_loss$$

where $propfruits_{HtoL}$ is the proportion of fruits that transitioned from healthy to lost.

For this calculation we again used means from the two extreme scenarios. This index assumes that: 1) healthy fruits do not abscise in the absence of frugivores, 2) infections by decomposers severe enough to result in loss were visible in the previous week, and 3) frugivores do not consume unhealthy fruit.

Plant-level analyses. We evaluated whether the number of fruits on the plant affected the rate of removal of individual fruits by using plant level data and running an ANOVA that included site-year combination and Julian date in the model. To evaluate whether the probability of any losses increased with fruit number we ran logistic regressions with some loss or no loss as the response variable and site-year combination, Julian date, and number of fruits as the predictors.

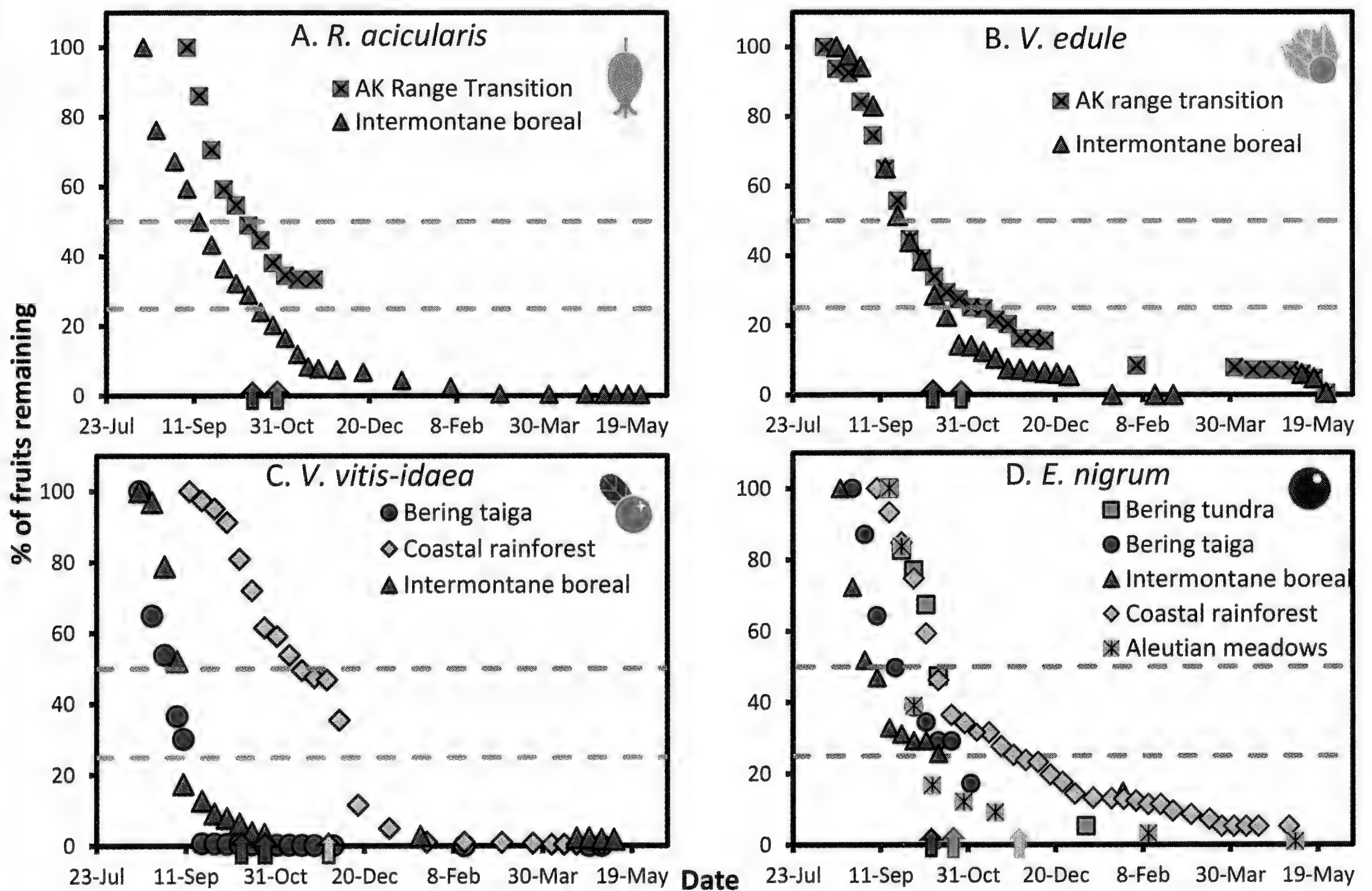


FIG. 3. Fruit loss over time for each species. Each data point is at the midpoint of the time period (a week in fall and spring, 2–4 wk in winter). The earliest date of collection was set to 100%, and each subsequent data point was calculated based on the mean percent fruit loss per day during the period for all site-year combinations in that ecoregion. This method was used because fruit collection was initiated on different dates across years and sites, so the actual percentage of the cohort remaining could not be compared across sites or years. Arrows along the x-axis indicate the long-term average time of snowfall, for most regions defined as from >50 mm of snowfall after the minimum daily temperature < 0°C until the mean daily temperature > 0°C but as December 1 for the Coastal Rainforest and Aleutian Meadows habitats.

RESULTS

General Patterns Across the Year

Numbers of fruits on the plants dropped steadily over the fall period for all four species, and most species in most ecoregions retained 25–50% of fruits by the date of season-long snow fall based on long-term averages (Fig. 3). For *V. vitis-idaea*, however, less than 25% of fruit was retained by start of snowfall in Bering Taiga and Intermontane Boreal ecoregions (Fig. 3C). In general, data collection was initiated earlier in northern ecoregions (Bering Taiga, Intermontane Boreal) than in southern ecoregions (Coastal Rainforest, Aleutian Meadows) (Fig. 3).

Comparisons Between Seasons

Collection periods for all three seasons varied widely by location and collecting group (Table 2), and number of sites for which data were available declined with each progressive season. For all four species, percent fruit lost was lower in spring than in the other two seasons (Fig. 4A–D). However, because the winter period was much longer than

the other two periods, the absolute loss rate (fruits lost per day per plant) was significantly lower in winter than in fall for all four species (Fig. 4E–H, Table 2). The only other seasonal differences were a higher absolute loss rate in fall than in spring for *E. nigrum* and in spring than in winter for *V. edule* (Table 2). The relative loss rate (% fruits lost per plant per day) was also higher in fall than in winter for all species except *R. acicularis*, but similar between fall and spring (and higher in spring for *V. edule*) (Fig. 4I–L, Table 2). In other words, for most species the relative rates of loss decreased during the period of snow cover, but returned in the spring to rates similar to or higher than those in fall.

The percent of fruits in a “healthy” state at the end of the fall varied by species (Fig. 4M–P). For *R. acicularis* only a small portion of the fruits remaining on the plants were still healthy at the time of snow fall (<30% for most sites; Fig. 4M) and this declined to almost zero in winter and spring. When comparing sites where both values were available, in fall plants had a higher percentage of healthy fruits than either winter or spring (Table 2). In sharp contrast, the vast majority of fruits of *V. vitis-idaea* and *E. nigrum* were in a healthy state at the time of snowfall (Fig. 4O, P),

TABLE 2. COMPARISON OF SEASONS. “Season” and “Season length” include all sites, while season comparisons include only sites with data for the two seasons being compared. Positive effect sizes indicate higher values for the first season listed. Rosaci = *Rosa acicularis*, Vibedu = *Viburnum edule*, Vacvit = *Vaccinium vitis-idaea*, Empnig = *Empetrum nigrum*. Values in bold indicate significant differences following a Benjamini-Hochberg procedure with a false discovery rate of 0.05.

Species	Season (# site-year combos)	Season length in days (range)	Comparison	Season Comparisons											
				# fruits lost plant ⁻¹ day ⁻¹				% fruit lost plant ⁻¹ day ⁻¹				% fruit in healthy state			
				Effect size	n	χ ²	P	Effect size	n	χ ²	P	Effect size	n	χ ²	P
Rosaci	Fall (33)	45 (12–92)	Fall vs winter	0.22	24	5.73	0.017	0.22	20	2.62	0.106	0.31	32	13.61	< 0.001
	Winter (12)	190 (148–228)	Winter vs. spring	<0.01	12	<0.01	0.999	–0.52	8	15.37	< 0.001	–0.01	26	1.06	0.30
	Spring (6)	24 (14–44)	Fall vs. spring	0.89	12	4.59	0.032	0.62	8	0.970	0.325	0.31	30	12.13	0.005
Vibedu	Fall (30)	49.0 (15–92)	Fall vs. winter	0.30	28	11.67	< 0.001	0.81	28	36.70	< 0.001	0.19	36	8.88	0.003
	Winter (15)	166.7 (42–203)	Winter vs. spring	–0.44	16	5.41	0.020	–0.51	16	6.11	0.013	–0.13	28	6.85	0.032
	Spring (9)	24.1 (6–46)	Fall vs. spring	–0.14	16	1.19	0.276	0.35	16	4.27	0.039	0.38	26	9.57	0.002
Vacvit	Fall (16)	45.6 (20–79)	Fall vs. winter	0.73	18	16.89	< 0.001	0.48	18	15.74	< 0.001	0.40	18	3.21	0.073
	Winter (9)	152.4 (76–212)	Winter vs. spring	0.10	10	0.06	0.800	–0.11	10	0.10	0.752	0.26	14	1.47	0.226
	Spring (5)	32.2 (5–80)	Fall vs spring	0.66	10	2.51	0.112	0.29	10	0.83	0.364	0.69	14	6.28	0.012
Empnig	Fall (14)	51.9 (13–114)	Fall vs Winter	0.88	14	16.73	< 0.001	0.32	14	8.22	0.004	0.58	14	6.69	0.010
	Winter (7)	145.7 (76–241)	Winter vs. spring	–0.20	8	0.895	0.344	–0.35	8	1.253	0.263	0.18	10	2.74	0.10
	Spring (4)	34.8 (4–80)	Fall vs. spring	0.40	8	14.50	< 0.001	0.63	8	0.31	0.578	0.99	10	33.65	< 0.001

and by the end of winter the variance was very high: many sites had no healthy fruits but at other sites, even within the same ecoregion, all or almost all fruits were in a healthy state. *Viburnum edule* showed an intermediate pattern, with a small, but significant, decline in percentage of healthy from fall to winter (Fig. 4N, Table 2).

Changes over the Fall Season

All species showed a drop in the number of fruits lost per day (numlost) over the course of the fall (Table 3, Fig. 5; statistically significant for all species after applying the Benjamini-Hochberg correction except *V. edule*). For *R. acicularis* there was an interaction between Julian date and ecoregion: there was a rapid decrease in fruits lost per day in the Alaska Range Transition sites but no change in the Intermontane Boreal sites (Fig. 5A, Table 3). Summer-cold sites (high PC2 sites) showed a steeper decline in fruits lost per day than summer-warm sites (low PC2 scores; Fig 6A). For *V. vitis-idaea*, winter-warm sites (low PC1 scores) had lower values than winter-cold sites (high PC2 scores); this pattern was driven by the communities of Pilot Point and Sitka (Fig. 6B). For *E. nigrum*, summer-cold sites (low PC2 scores) had higher rates of loss than winter-cold sites (high PC2 scores; Fig. 6C).

Across all sites, the relative loss rate (perlost) was constant over the course of the fall for all species except *E. nigrum*, where it declined over time (Table 3, Fig. 7). However, for some species there were opposing patterns by ecoregion (a significant interaction between Julian date and ecoregion). In *R. acicularis* the relative loss rate declined throughout fall in Alaska Range Transition sites but showed no change for the Intermontane Boreal sites (Fig. 7A). Winter-warm sites showed steeper declines than winter-cold sites, with positive slopes (higher perlost as the season progressed) for the coldest sites (Fig. 6D). *Vaccinium vitis-idaea* also showed significant differences in perlost between ecoregions, with lower relative loss rates in Coastal Rainforest than in the Bering Taiga and Intermontane Boreal ecoregions (Fig. 7C, and, consistent with this, lower loss rates in winter-warm (low PC2) sites, driven by Pilot Point and Sitka (Fig. 6E). *Empetrum nigrum* showed no differences between ecoregions (Fig. 7D), but summer-warm sites had lower loss rates than summer-cold sites (Fig. 6F).

Despite these general patterns of consistent loss throughout the fall, observers reported pulses in fruit loss at individual sites. Sharp declines in the number of healthy fruits between observation periods were paired with observations of animal activity: a 23% drop in one week for *V. edule* site in Two Rivers (Intermontane Boreal) in 2017 and a 15% drop in one week in *V. vitis-idaea* in Shageluk (Bering Taiga) attributed to grouse, a 20% drop in one week in *R. acicularis* in Venetie (Intermontane Boreal) in 2016 attributed to snowshoe hares and a damage rate of

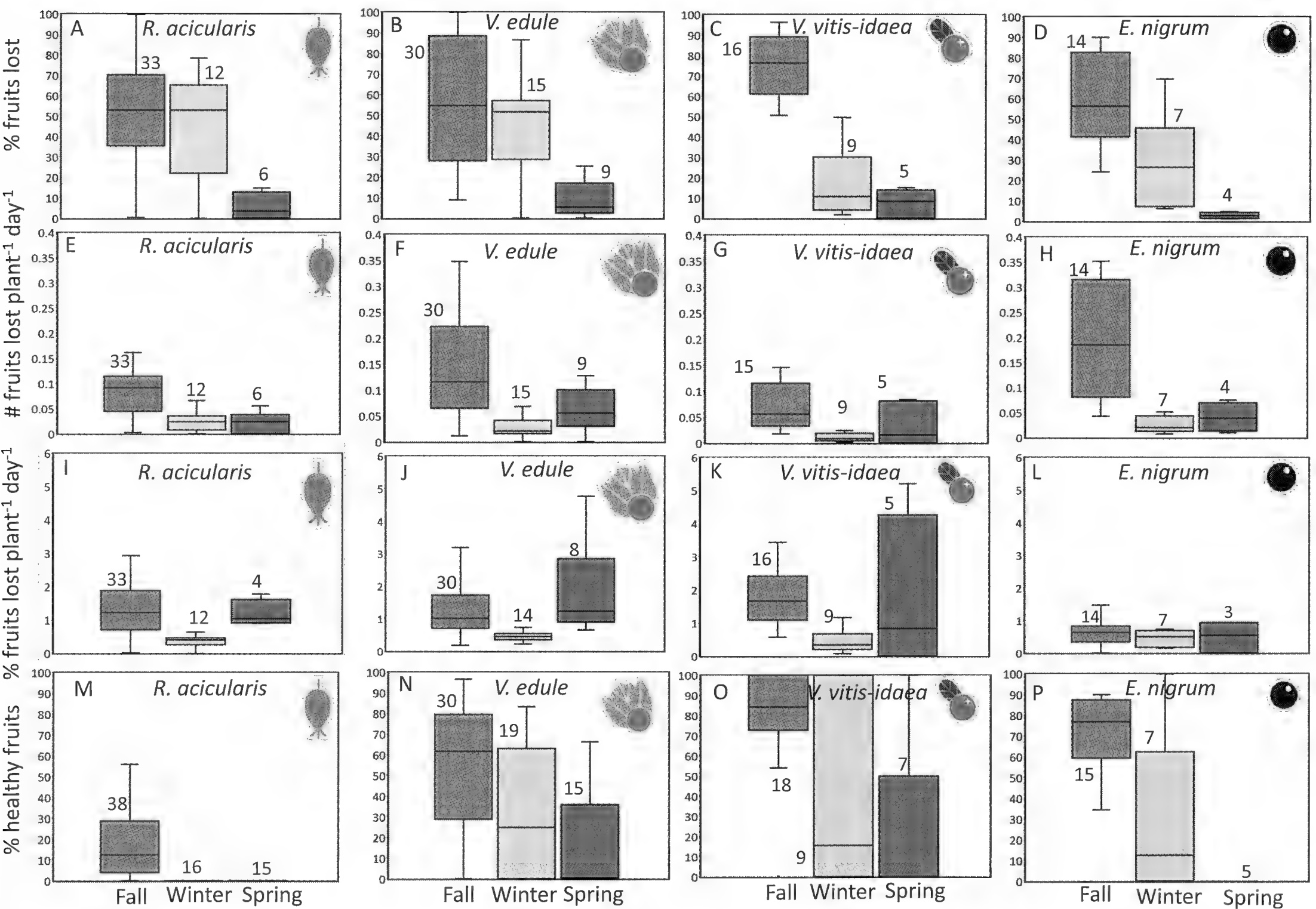


FIG. 4. Seasonal differences. A–D. Percent fruits lost during the season. Because sample size varied by season, values do not sum to 100. E–H. Number of fruits lost per plant and per day. I–L. Percent of fruits lost per plant and per day. M–P. Percent of the fruits remaining at the end of the season that are healthy. Samples sizes are number of year-site combinations. Graphs do not match results in Table 2 exactly because they show averages for all sites, whereas in Table 2 comparisons are limited to sites where data for both years were available.

75% of *V. edule* in one week by an unknown invertebrate in Palmer (Alaska Range Transition) in 2018. Smaller losses (5–10% in one week) were also associated with observed high levels of activity of bear for *R. acicularis* in Holy Cross (Bering Taiga) in 2018, and migratory waterfowl for *E. nigrum* in Scammon Bay (Bering Taiga) in 2018. However, in general pulse events were rare: >15% loss in one week were observed once for *V. vitis-idaea* and *V. edule* (out of 148 and 250 weekly observations resp.)

TABLE 3. EFFECT OF JULIAN DATE, ECOREGION, AND THEIR INTERACTION ON NUMBER OF FRUITS LOST PER DAY (NUMLOST), PERCENT OF FRUIT LOST PER DAY (PERLOST), AND PERCENT OF FRUIT THAT ARE HEALTHY (PERHEALTHY) IN FALL. Values in bold indicate significant differences following a Benjamini-Hochberg procedure with a false discovery rate of 0.05.

Species	Explanatory variable	numlost				perlost				perhealthy			
		χ^2	df	P	N	χ^2	df	P	N	χ^2	df	P	N
<i>R. accularis</i>	Julian date	21.31	1	<0.001	190	2.81	1	0.09	199	126.2	1	<0.001	268
	Ecoregion	1.15	1	0.563		<0.01	1	0.95		6.77	1	0.009	
	Interaction	8.46	1	0.003		7.62	1	0.006		4.38	1	0.036	
<i>V. edule</i>	Julian date	4.30	1	0.038	182	0.15	1	0.698	181	50.14	1	<0.001	218
	Ecoregion	<0.01	1	0.991		0.23	1	0.635		0.35	1	0.553	
	Interaction	0.76	1	0.383		1.04	1	0.308		4.54	1	0.033	
<i>V. vitis-idaea</i>	Julian date	7.71	1	0.005	97	0.04	1	0.844	102	22.08	1	<0.001	123
	Ecoregion	5.50	2	0.063		10.68	2	0.005		1.31	2	0.517	
	Interaction	1.33	2	0.515		1.54	2	0.463		2.56	2	0.278	
<i>E. nigrum</i>	Julian date	12.11	1	<0.001	74	6.09	1	0.014	76	34.70	1	<0.001	92
	Ecoregion	7.58	4	0.108		3.08	4	0.544		12.09	4	0.017	
	Interaction	4.40	4	0.354		1.46	4	0.834		9.63	4	0.047	

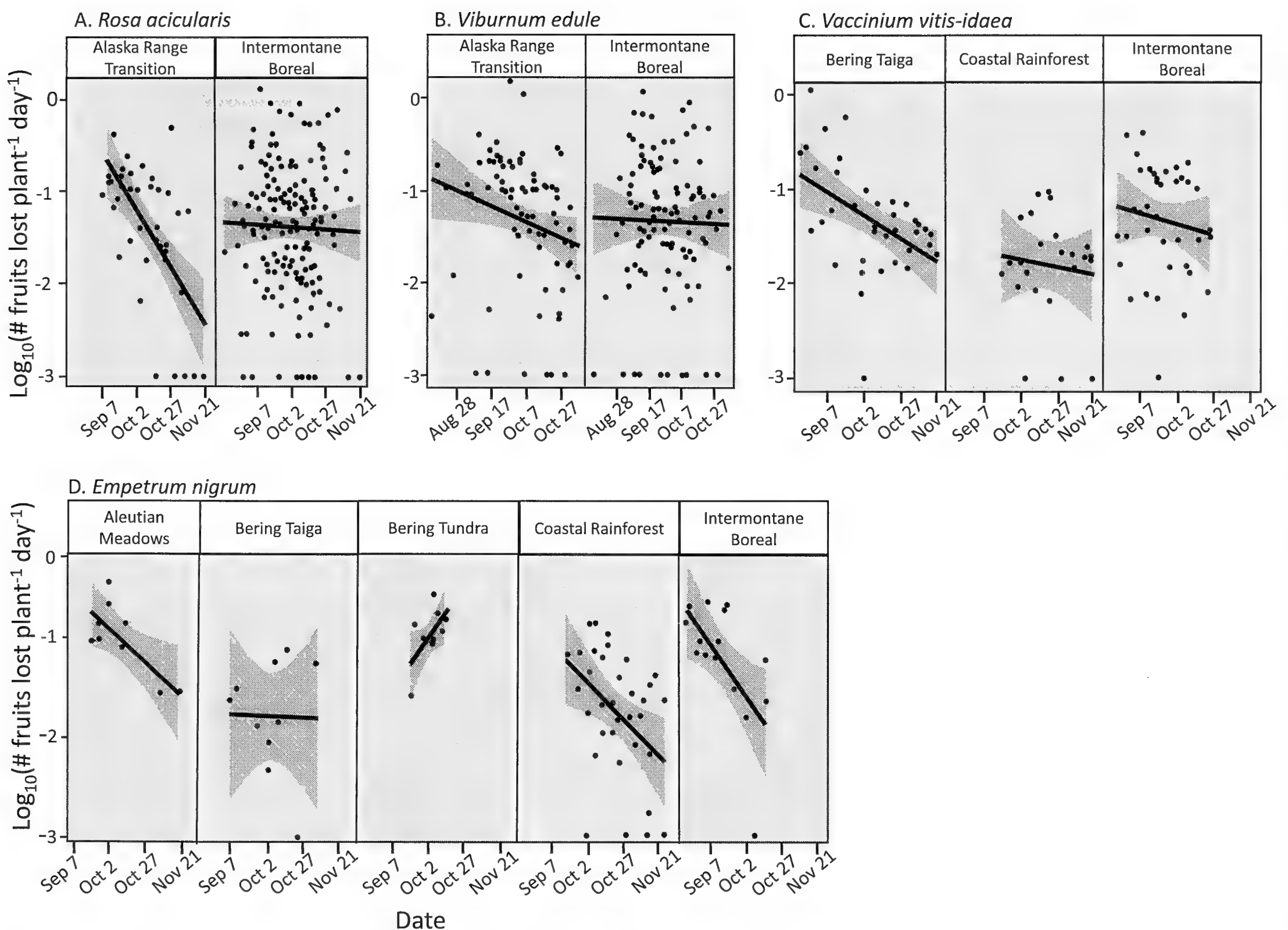


FIG. 5. Number of fruits lost per plant and per day by region and by Julian date. Data points are site means. Shaded area indicates the 95% confidence interval around the regression line. Results of the analyses can be found in Table 3.

and twice for *R. acicularis* (out of 201 observations); there were none for the 119 *E. nigrum* observations.

The proportion of berries on the plant that were healthy declined over the course of the fall for all four species (Table 3, Fig. 8). For *R. acicularis* there was a significant difference between ecoregions, with a lower percentage of healthy in the Intermontane Boreal ecoregion than in the Alaska Range Transition (Table 3, Fig. 8A). However, winter-warm sites had steeper declines than winter-cold sites over the course of the fall (Fig. 6G). For *V. vitis-idaea* and *V. edule* there were no differences between ecoregions (Fig. 8C) and climate variables did not explain significant amounts of variation in this trait ($P > 0.1$ for all). For *E. nigrum*, there were differences in means, with the lowest percentage of healthy fruits in the Aleutian Meadows ecoregion (Fig. 8D), but no differences in slopes (Table 3). Winter-warm sites (Sitka, Homer, and Unalaska) had lower percentage of healthy fruits than winter-cold sites (Fig. 6H).

Transitions Between States

Comparisons of species. When we evaluated transitions of individual fruits between states (healthy, unhealthy, or lost), the proportion of

healthy fruits lost per interval (usually a week) was smaller than the proportion of unhealthy fruits lost in both fall and spring for all four species (Table 4). In fall, *R. acicularis* had the highest rate of transition from healthy to unhealthy, but a low rate of unhealthy fruit loss; multiplying these two rates resulted in the highest rate of healthy fruits that were lost following infection / dehydration (1.8% per wk; Table 4). *Empetrum nigrum* had a similar rate of healthy fruits lost following infection / dehydration (1.8% per wk) but this was driven primarily by high loss rates of unhealthy fruits rather than high rates of transition from unhealthy to healthy. *Viburnum edule* was intermediate in both rates of transition from healthy to unhealthy and loss rates of healthy fruits, resulting in intermediate loss rates of healthy fruits following infection / dehydration (0.7% per wk). *Vaccinium vitis-idaea* had the highest loss rates of unhealthy fruits, but the rates of transition from healthy to unhealthy were very low so the rate of loss of healthy fruits following infection / dehydration was also very low (0.4% per wk). Our index of the relative importance of vertebrate frugivory (the ratio of direct loss of healthy fruits to indirect losses via the unhealthy state) was >6 for all species, lowest for *R. acicularis* and highest for *V. vitis-idaea* in both fall and winter (Table 4).

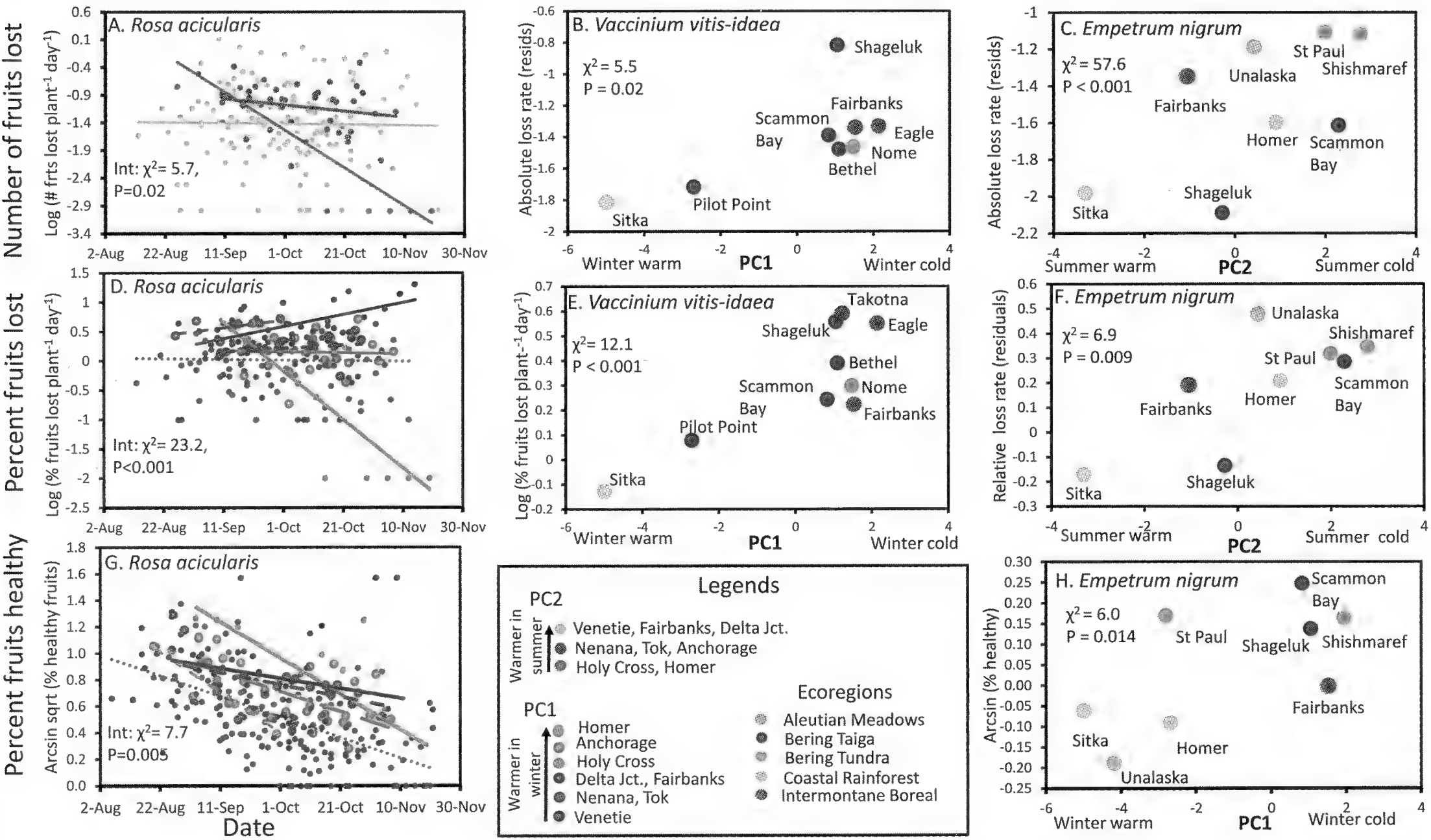


FIG. 6. Changes in fruit loss and percent healthy fruits by climate gradient. Only variables that showed a relationship between a response variable and PC1 or PC2 (panels B,C,E,F and H) or significant interactions between Julian date and PC1 or PC2 (panels A,D,G) are shown. For *R. acicularis* (A,D, and G) there were no significant main effects of PC1 and PC2. For absolute loss rates (panels B and C) and relative loss rates in *E. nigrum* (panel F), values are residuals after including Julian date in the model (for effects of Julian date, see Table 3).

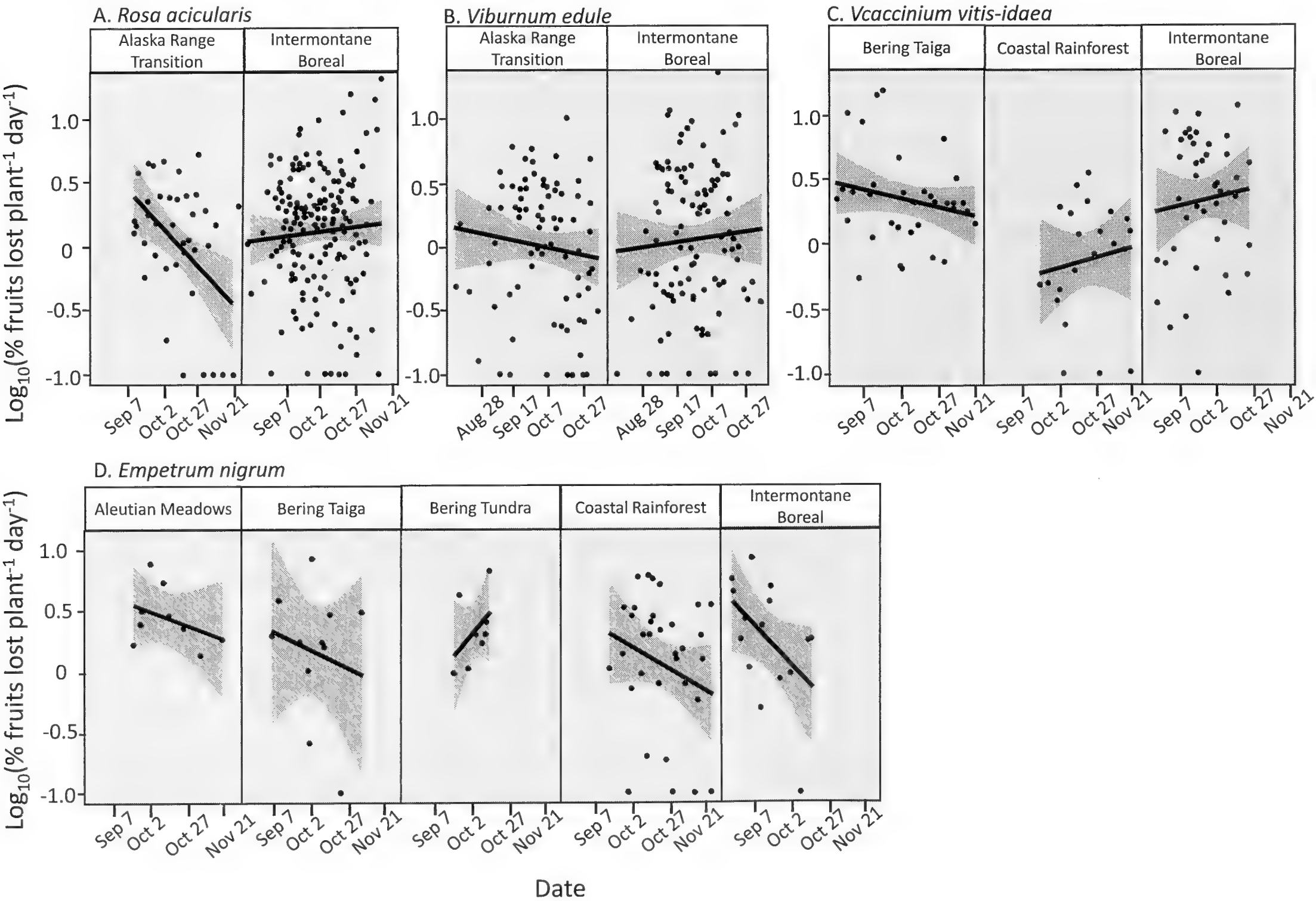


FIG. 7. Percent of fruits lost per plant per day by ecoregion and Julian date. Datapoints are site means. Shaded area indicates the 95% confidence interval around the regression line. Results of the analyses can be found in Table 3.

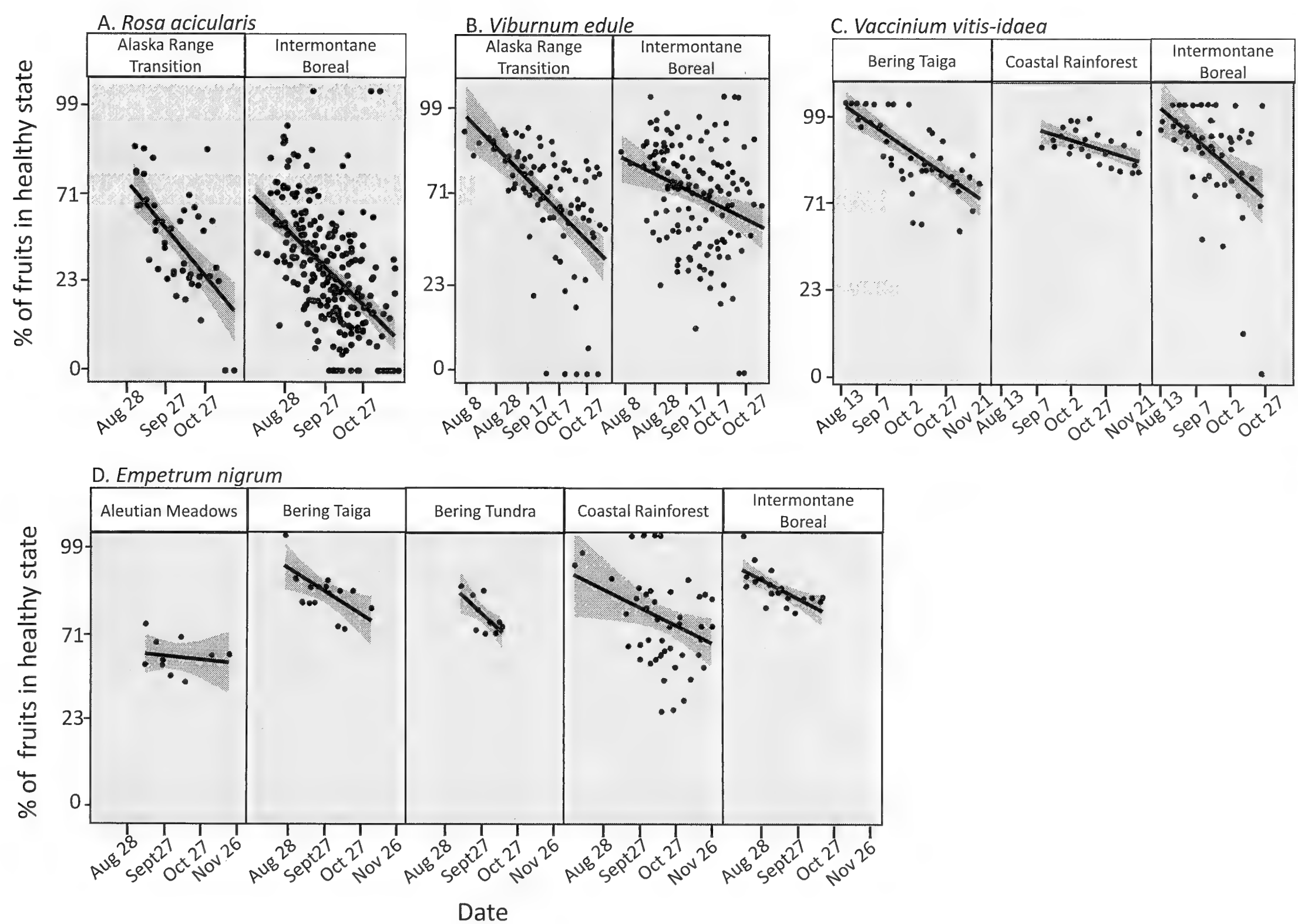


FIG. 8. Changes in percentage of fruits in a healthy state over the course of the fall period. Data points are site means. Values on the y-axis are back-transformed from an arcsin square-root transformation; the axis is not linear. Shaded area indicates the 95% confidence interval around the regression line. Results of the analyses can be found in Table 3.

Comparisons of seasons. The proportion of fruits that remained healthy from week to week was reduced between fall and spring, though this reduction was much greater for *R. acicularis* and for *E. nigrum* than for the other two species (Table 4). For three out of four species this was primarily because a higher proportion of fruits went to an unhealthy state, but for *E. nigrum* it was the result of

TABLE 4. PROPORTIONS OF FRUITS TRANSITIONING BETWEEN STATES FOR CONSECUTIVE OBSERVATIONS BY SEASON. Ranges are based on the two scenarios that differ in how they deal with ambiguous transitions (losses attributed first to healthy fruits vs. first to unhealthy fruits).

Species (# plant observations)	Season (# fruit observations)	State at start of interval	State at end of interval			Prop. healthy fruits lost via unhealthy fruits per week	Ratio of direct to indirect loss
			Healthy	Unhealthy	Lost		
<i>Rosa acicularis</i> (2601)	Fall (15,640)	Healthy	0.67	0.14–0.24	0.09–0.18	0.018	7.5
		Unhealthy	0	0.71–0.76	0.15–0.24		
	Spring (408)	Healthy	0.22	0.26–0.35	0.28–0.35	0.043	7.3
		Unhealthy	0	0.71–0.72	0.28–0.29		
<i>Viburnum edule</i> (3085)	Fall (29,691)	Healthy	0.82	0.07–0.09	0.10–0.12	0.007	15.7
		Unhealthy	0	0.79–0.86	0.14–0.21		
	Spring (713)	Healthy	0.74	0.16–0.18	0.08–0.10	0.031	2.9
		Unhealthy	0	0.64–0.64	0.36–0.36		
<i>Vaccinium vitis-idaea</i> (2237)	Fall (7681)	Healthy	0.80	0.02–0.02	0.18–0.18	0.004	45
		Unhealthy	0	0.60–0.66	0.34–0.40		
	Spring (84)	Healthy	0.77	0.03–0.04	0.19–0.2	0.004	48.8
		Unhealthy	0	0.74–0.77	0.23–0.26		
<i>Empetrum nigrum</i> (1915)	Fall (4984)	Healthy	0.77	0.06–0.08	0.15–0.18	0.018	9.2
		Unhealthy	0	0.61–0.71	0.28–0.38		
	Spring (51)	Healthy	0.18	0.18–0.18	0.64–0.64	0.023	27.8
		Unhealthy	0	0.75–0.75	0.25–0.25		

TABLE 5. PROPORTIONS OF FRUITS TRANSITIONING BETWEEN STATES FOR CONSECUTIVE OBSERVATIONS IN FALL BY ECOREGION. Ranges are based on the two scenarios that differ in how they deal with ambiguous transitions (ambiguous losses attributed to healthy fruits vs. to unhealthy fruits).

Species (# plant observations)	Ecoregion (# fruit observations)	State at start of interval	State at end of interval			Prop. healthy fruits lost via unhealthy fruits per week	Ratio of direct to indirect loss
			Healthy	Unhealthy	Lost		
<i>Rosa acicularis</i> (2601)	Bering Taiga (381)	Healthy	0.62	0.18–0.25	0.14–0.20	0.031	5.5
		Unhealthy	0	0.67–0.76	0.24–0.33		
	Intermontane Boreal (9590)	Healthy	0.66	0.14–0.26	0.08–0.20	0.027	37.0
		Unhealthy	0	0.75–0.84	0.16–0.25		
	AK Range Transition (3039)	Healthy	0.73	0.13–0.18	0.09–0.14	0.013	68.7
		Unhealthy	0	0.81–0.85	0.14–0.19		
<i>Viburnum edule</i> (3068)	Intermontane Boreal (15,001)	Healthy	0.81	0.05–0.08	0.11–0.14	0.008	15.6
		Unhealthy	0	0.79–0.86	0.14–0.21		
	AK Range Transition (14,690)	Healthy	0.84	0.07–0.09	0.08–0.10	0.007	12.9
		Unhealthy	0	0.83–0.88	0.16–0.17		
	Bering Taiga (1623)	Healthy	0.81	0.02–0.02	0.16–0.16	0.004	40
		Unhealthy	0	0.60–0.62	0.38–0.40		
<i>Vaccinium vitis-idaea</i> (1913)	Intermontane Boreal (2601)	Healthy	0.74	0.01–0.03	0.23–0.24	0.009	26.1
		Unhealthy	0	0.47–0.59	0.41–0.53		
	Coastal Rainforest (1662)	Healthy	0.90	0.01–0.01	0.09–0.09	0.001	90
		Unhealthy		0.86–0.86	0.14–0.14		
	Bering Tundra (833)	Healthy	0.81	0.05–0.09	0.10–0.15	0.012	14.6
		Unhealthy	0	0.59–0.74	0.26–0.41		
<i>Empetrum nigrum</i> (1215)	Bering Taiga (280)	Healthy	0.81	0.04–0.06	0.15–0.15	0.010	15.0
		Unhealthy	0	0.60–0.64	0.36–0.40		
	Intermontane Boreal (893)	Healthy	0.78	0.03–0.08	0.17–0.19	0.009	20.0
		Unhealthy	0	0.48–0.73	0.27–0.39		
	Aleutian Meadows (414)	Healthy	0.63	0.11–0.21	0.16–0.26	0.035	5.1
		Unhealthy	0	0.50–0.64	0.38–0.50		
	Coastal Rainforest (1084)	Healthy	0.76	0.05–0.07	0.16–0.17	0.007	23.6
		Unhealthy	0	0.75–0.78	0.21–0.25		

greater losses of healthy fruits from the plant. For *R. acicularis* and *V. edule* the proportion of unhealthy fruits that were lost in spring vs. fall was higher, for *E. nigrum* the values were similar, and for *V. vitis-idaea* they were lower. As a result, the ratio of direct losses of healthy fruits to indirect losses via unhealthy fruits was much lower in spring than in fall for *R. acicularis* and *V. edule*, similar for *V. vitis-idaea*, and higher for *E. nigrum*.

Comparisons of ecoregions. In general, the more northern ecoregions (Bering Tundra, Bering Taiga, and Intermontane Boreal) had lower rates of loss for unhealthy fruits than the more southern regions (Alaska Range Transition, Coastal Rainforest, and Aleutian Meadows), driving lower indirect losses of healthy fruits via an unhealthy state, though for *V. edule* the two ecoregions were very similar (Table 5). An exception was for *E. nigrum* in the Aleutian Meadows, which had very high rates of loss of healthy fruits. The ratio of direct to indirect losses of healthy fruits showed the expected inverse pattern (Table 5).

Plant Level Effects

For all four species plants with more fruits were more likely to have at least one fruit removed (*R. acicularis*: $Z = 7.12$, $P < 0.001$, parameter estimate

[PE] = 0.088 ± 0.011 ; *V. edule*: $Z = 8.36$, $P < 0.001$, PE = 0.046 ± 0.005 ; *V. vitis-idaea*: $Z = 6.47$, $P < 0.001$, PE = 0.160 ± 0.025 ; *E. nigrum*: $Z = 4.69$, $P < 0.001$, PE = 0.091 ± 0.020). For *R. acicularis*, *V. vitis-idaea* and *E. nigrum* the proportion of fruits removed was not affected by the number of fruits on the plant ($F_{(1,232)} = 0.026$, $P = 0.96$, $F_{(1,1899)} = 3.47$, $P = 0.06$, and $F_{(1,1197)} = 2.32$, $P = 0.13$ respectively), but for *V. edule* a higher proportion of fruits were removed from plants with more fruits ($F_{(1,2442)} = 6.55$, $P = 0.01$, PE = 0.00186 ± 0.00072).

DISCUSSION

Through a very high quality, robust, geographically diverse dataset collected by ≈ 1500 volunteers across Alaska, this study provides baseline natural history of fruit retention and fate over time for the four focal species. Following ripening in August, all four species showed a reduction in absolute loss rates over the course of the fall; since the majority of these fruits were in a healthy state, this indicates that the supply rate to frugivores diminished over the course of the fall. For two species, *V. edule* and *V. vitis-idaea*, the relative loss rate (% fruits lost per day) did not change over time, indicating that an individual fruit is as likely to be lost in early fall as in late fall. This pattern of constant loss rate explains why plants invest in persistent fruits and is similar to that for

Ilex verticillata (L.) A.Gray and *Mitchella repens* L. in Maine (Gervais and Wheelwright 1994), but in sharp contrast to *C. canadensis*, which showed a rapid loss of the majority of fruits due to migratory birds in late fall (Burger 1987), and *V. opulus*, which lost fruit rapidly in late November, in part due to abscission by the parent plant (Gervais and Wheelwright 1994). *Rosa acicularis* showed a steep decline in proportional loss rates over time in the Alaska Range Transition sites, but no change in the Intermontane Boreal ecoregion, while *E. nigrum* showed a decline in proportional loss rates in all habitats. Observers reported five events with rapid fruit loss or damage (pulse events) associated with four different animals in four ecoregions, and our data showed high rates of loss (>15% in a week) on four occasions. While these events were dramatic (especially for the youth and adults observing them!), these occurrences represent <5% of the 106 yr-site combinations. Our sites were not randomly located across the state and were all located near towns or villages, and it is possible that this resulted in reduced frugivore populations and is partially responsible for low pulse events. However, small species like grouse, red-backed voles and snowshoe hare are common at the sites used in this study (e.g., there were many reports of sightings of voles and hares by observers), and even large vertebrates such as bears (e.g., Smith et al. 2005) and foxes (Selås et al. 2010) may become habituated to and even attracted to areas of human habitation (sign of one bear and multiple foxes were also observed). While we cannot conclude that pulse events are uncommon, our dataset provides no evidence that they are common.

During the winter period the absolute rates of fruit loss were reduced compared to spring in all species and the relative rate was reduced in all species except *R. acicularis*. The continued high percent fruit removal in *R. acicularis* is likely the result of resident winter birds and snowshoe hare, as suggested by observer sightings of these animals or their sign in or near the sites. The two short-statured ericaceous species had a smaller reduction in relative loss rates in winter than *V. edule* (Table 2); the most likely explanation is continued frugivory by subnivean species such as voles (West 1982; Krebs et al. 2010). In spring the relative rates of fruit loss returned to rates similar to those in fall (though absolute rate was higher for *E. nigrum*). However, whether the losses were driven by healthy or unhealthy fruits depended on the species; we discuss this further in the next section.

Frugivores Versus Decomposers

While we did not measure losses to frugivores vs. decomposers directly, our estimates of transitions between states (healthy, unhealthy, and lost) allow us to draw some inferences. Based on casual observations we expected the highest rates of loss to decomposers in *R. acicularis* and *V. edule* which

carry high proportions of infected fruit (Fig. 1A, B), and the lowest rates for the two ericaceous species, *V. vitis-idaea* and *E. nigrum*, on which infected fruits are seldom seen. These observations were confirmed by the Winterberry data: at the end of the fall *R. acicularis* had the lowest percentage of healthy fruits while *V. vitis-idaea* and *E. nigrum* had the highest percentage of healthy fruits (>80% for both) while *V. edule* was intermediate (Fig. 4M–P). However, the data on transitions between states in fall revealed that the percentage of unhealthy fruits on the plant is not a good indication of the relative importance of decomposers because species differ in the rate at which infected fruits are lost from the plant. As expected, *R. acicularis* had the highest rates of healthy fruits lost following infection/dehydration ($\approx 2\%$ per wk). At the other extreme, *V. vitis-idaea* had by far the lowest rates of fruits lost following infections (0.4% per wk), and this was driven by the very low transition of fruits from healthy to unhealthy fruits. *Vaccinium* species are protected by high levels of organic acids and phenolics and by the presence of protective nonpathogenic fungi (Cipollini and Stiles 1992, 1993; Aiken et al. 2007; Ermis et al. 2015; Stobnicka and Gniewosz 2017). However, once infected the fruits were dropped rapidly (34–40% per wk), contributing to the low conspicuousness of infected fruits. Fruits of *V. edule* and *E. nigrum* were about equally likely to become unhealthy, but there were very few unhealthy *E. nigrum* fruits on the plants because they were abscised at high rates, while unhealthy *V. edule* fruits are common because they are retained on the plant.

If we assume that losses of healthy fruits are due to vertebrate frugivores and losses of unhealthy fruits are due to decomposers and invertebrates, then in fall, frugivores removed 6–45 times as many fruits as decomposers/invertebrates, with the lowest ratio for *R. acicularis* and the highest for *V. vitis-idaea*. It is possible that a few fruits became unhealthy and dropped within an observation interval, or that healthy fruits are abscised. We consider the latter unlikely: in the pilot year we tracked fruits on the ground as well as on the plant and observed very few healthy fruits on the ground (Mulder unpublished data). It is more likely that some unhealthy fruits are consumed by vertebrate frugivores, especially as healthy fruit becomes scarce (García et al. 1999). However, we have several reasons to believe unhealthy fruits are likely unpalatable or less palatable to vertebrate frugivores. First, fruits that are in an infected or shriveled state have lower dry mass than “healthy” fruits; for “rotten” and “dry” fruits collected in September of 2020 the reduction in dry mass was 37% and 48% for *V. edule* and 19% and 38% respectively in *R. acicularis* (Mulder unpublished data). Water content was also lower in “dry” fruits than in healthy ones (20% in both species; Mulder unpublished data). Unhealthy fruits are therefore likely of lower nutritional value to herbivores than healthy fruits. Second, fruits infected by

microbes or insects may be less palatable than uninfected fruits (e.g., Manzur and Courtney 1984; Burger 1987; Cipollini and Stiles 1993; Traveset et al. 1995; García et al. 1999). This begs the question: why do *R. acicularis* and *V. edule* retain unhealthy fruit for so long? Further research on the relative losses to vertebrate frugivores, invertebrate frugivores, and different groups of decomposers (e.g., fungi and bacteria) using approaches such as exclosure experiments and camera traps are needed to understand the fate of fruits and their seeds in these habitats.

Invertebrate damage. Damage by invertebrates appeared to be low in the two ericaceous species, with reports of ants on fruits in one Intermontane Boreal site for each species, and one report of a snail on *Empetrum nigrum* in the Aleutian Meadows. Damage by invertebrates may be quite high in *V. edule*, where the $\approx 15\%$ of fruits classified as “dry” by observers at the end of fall frequently appeared to have intact integument and seed but no pulp. Burger (1987) observed that on *Cornus canadensis*

“...slugs made small holes in the fruit integument and then ate out much of the inner flesh leaving the seed and skin attached to the plant”.
(p. 6)

We did receive reports of invertebrates on *V. edule* including the sighting of one caterpillar, reports of spider webs covering branches at three Alaska Range Transition and two Intermontane Boreal sites, and several “stink bugs” present on fruits, suggesting invertebrate frugivory a likely explanation for these flat fruits. Observers at seven sites noted “punctures” in fruits of *R. acicularis*, and there were at least six reports of damage followed by “rotting”. It seems likely that in this species invertebrate damage increases the probability of infection by microbes.

Differences between fall and spring. We had expected high fruit loss rates in spring due to snowmelt providing greater access to fruits, warmer conditions increasing decomposition rates, and the reappearance of hibernating or migrating animals. While all fruits had higher relative fruit loss in spring than in fall, the main drivers differed among species. We found support for higher frugivory rates in spring for only one species: in *E. nigrum* healthy fruits were lost at almost quadruple the rate in fall, but unhealthy loss rates were unchanged, which we interpret as preferential removal by animals. We found support for higher spring decomposition rates in two species: *R. acicularis* had a tripling from fall to spring in the rate of healthy fruit transitioning to unhealthy and in *V. edule* loss rates of unhealthy fruits doubled. However, higher losses of unhealthy fruits could also be the result of increased reliance on sub-optimal fruit by frugivores as food becomes scarce (e.g., Foster 1977; Stiles 1980). The fourth species, *V. vitis-idaea*, showed very little change from fall. These identity-dependent changes between sea-

sons suggest it is difficult to extrapolate from our results to other species with persistent fruits.

Differences Between Ecoregions

We had expected higher loss rates in the more southern ecoregions: they are warmer, wetter, and have a longer snow free period (Fig. 2B). These predictions were not supported; *V. edule* showed no differences in loss rates between ecoregions and no relationships with the climate variables. *Empetrum nigrum* showed no differences between ecoregions, no relationship with winter conditions (PC1), and the relationship with the summer conditions axis was the opposite of that expected: the warmest sites had the lowest fruit losses. *Vaccinium vitis-idaea* also showed the opposite pattern from the predicted one: it had a lower relative loss rate in the Coastal Rainforest sites than in other ecoregions, and lower absolute loss rates in winter-warm sites like Sitka and Pilot Point. *Rosa acicularis* was the only species where there was some evidence for greater loss at warmer sites: steeper absolute loss rates in summer-warm sites, and steeper relative loss rates in winter-warm sites (but no main effects of PC1 or PC2). In summary, for two species there was support for higher loss rates at colder sites, for one there was support for greater changes at warmer sites, and for one there was no support for differences by ecoregion or climate gradients. Lower loss rates in more southern or warmer regions for the two ericaceous species, which are small and have few fruits, may be the result of a greater variety of fruits available to vertebrate frugivores. Of 50 species of fleshy fruits in Hultén (1968), 43 can be found in the Coastal Rainforest, 38 in the Alaska Range transition, 27 in the Intermontane Boreal, 24 in the Aleutian Meadows, 19 in the Bering Taiga, and only 14 in the Bering Tundra. However, frugivory is also likely driven by the diversity and density of the frugivores. An investigation of competition for frugivores would help clarify the patterns we found.

There was also little evidence for higher rates of loss to decomposers in the more southern ecoregions. At time of snow fall the proportion of healthy fruits still available in southern ecoregions was either higher than (*R. acicularis*, *V. vitis-idaea*) or similar to (*V. edule*, *E. nigrum*) those in more northern ecoregions (Fig. 8). While this may be partially attributable to later ripening in the southern ecoregions than in the more northern ones, data on the fate of individual fruits also suggest a lower rate of loss to decomposers in the more southern regions. For *R. acicularis* and *V. vitis-idaea* the proportion of healthy fruits lost after transitioning to unhealthy was the lowest in the southern-most ecoregions, and for *V. edule* it did not differ. For *E. nigrum* this value was much higher in the Aleutian Meadows than in the other ecoregions (Table 5), and the three sites that were warmest in winter (Sitka, Unalaska, and Homer) had the lowest percentage of healthy fruits

(Fig. 6H). However, even those results are not clear, as for Sitka this was driven by a high retention of unhealthy fruit rather than a high rate of transition from healthy to unhealthy. These results are not easily explained and point to the need for a greater understanding of the microbiome of wild fruits.

Potential Consequences under Climate Change

Warmer temperatures will reduce the number of days with snow cover, and earlier springs are expected to lead to earlier flowering and fruiting in our focal species (Mulder and Spellman 2019). Given that for most species and most locations the probability of fruit loss was constant over the course of the fall, and that loss rates during the snow-free period was higher than during winter for all species, all else being equal we predict a lower number of fruits at time of snow fall and reduced food availability for frugivores in winter and spring in future decades. However, our study did point to the potential for some resilience: since a small proportion of fruits were still on the plant in April and May, there is the potential for southern genotypes to be transported to more northern latitudes during spring bird migration. The distance by which the average seed is transported in or on birds is unknown, but it is presumably larger than would normally occur in unaided migration. This may be important if southern genotypes are better adapted to the warmer conditions expected in northern latitudes. We are aware of only one study on genotypic variation or local adaption in our target species: Roy and Mulder (2014) conducted a common garden / reciprocal transplant experiment and found some evidence for differential survival and morphology of *V. vitis-idaea* genotypes from different origins, but little evidence for local adaptation. However, although selected to maximize differences in environmental conditions, the origin sites were located within 60 km of each other, and role of local adaptation over a larger scale is unknown.

CONCLUSIONS

This study is a first attempt at understanding the complexities of fruit loss for four plant species with persistent fruits. While the four species shared some patterns (e.g., similar overall patterns of change between seasons), they showed very different patterns of loss for healthy vs. unhealthy fruits and unexpected differences between ecoregions. The large spatial scale and large number of sites at which we obtained data allowed us to compare ecoregions and demonstrate that events like rapid fruit loss are uncommon. This work would not have been possible without an extensive community science network of dedicated group observers who are passionate about berries and demonstrates the value of public participation in scientific research.

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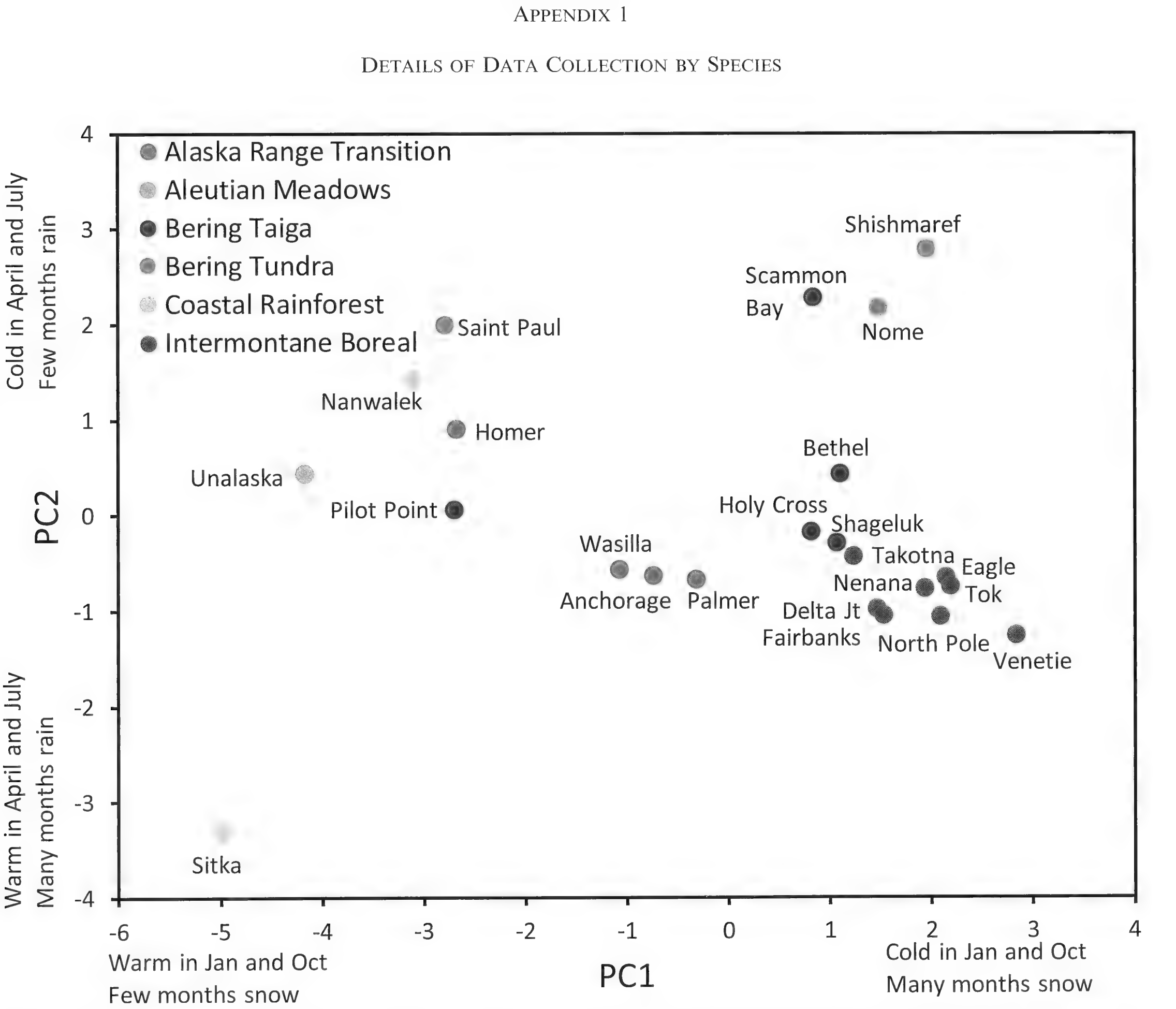
DATA ACCESSIBILITY

All data are available at <http://dx.doi.org/10.6073/pasta/6c5885f8f1423b274a3fea6c20e25c66>

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APPENDIX FIG. A. Communities by ecoregion and climate gradients. PC1 values (x-axis) indicate conditions in fall and winter while PC2 (y-axis) indicates conditions in spring and summer.

APPENDIX 1 TABLE A. *ROSA ACICULARIS*. An asterisk (*) signifies that a site is in the transition zone between Bering Taiga and Intermontane Boreal, but was combined with Intermontane Boreal due to low sample size.

Region	Site	Years	# Plants	# Dates
Intermontane Boreal	Holy Cross Elementary School*	2018–2019	19	7
	Anne Wien Elementary School	2018–2019	19	7
		2019–2020	30	7
	Arctic Light Elementary School	2017–2018	10	5
		2018–2019	6	3
	Boreal Sun Charter School	2019–2020	12	5
	Delta Future Farmers of America	2017–2018	20	5
		2018–2019	18	8
		2019–2020	12	8
	Denali Elementary School	2018–2019	16	11
		2019–2020	20	13
	Hunter Elementary School	2018–2019	22	2
	Mulder Yard	2016–2017	20	9
		2017–2018	20	4
		2018–2019	18	9
		2019–2020	20	8
	Murie Trail	2018–2019	14	2
	Nenana School	2018–2019	17	4
		2019–2020	15	5
	Parkinson Yard	2016–2017	20	16
	Parkinson Site 2	2016–2017	20	9
	Tanana Middle School	2016–2017	21	11
		2017–2018	18	6
	Tok School	2018–2019	20	4
	UAF Satellite Dish	2016–2017	20	5
	John Fredson School	2016–2017	21	5
		2018–2019	18	7
		2019–2020	12	12
	Watershed Kindergarten	2017–2018	11	5
		2018–2019	11	
	Watershed Powerline Trail	2017–2018	11	8
		2018–2019	8	3
		2019–2020	10	2
	Watershed Sit Spots	2017–2018	19	8
		2018–2019	8	6
		2019–2020	10	5
	Total	36	586	234
Alaska Range Transition	Center for Alaska Coastal Studies HQ	2019–2020	17	12
	Campbell Creek Bridge	2017–2018	20	4
	Polaris K–12 School	2017–2018	26	6
		2018–2019	18	8
		2019–2020	26	9
	Totals for Alaska Range Transition	8	166	39
Species total	23 sites	44	752	273

APPENDIX 1 TABLE B. *VIBURNUM EDULE*. An asterisk (*) indicates a site was on the edge of the Coastal Rainforest, but counted as Alaska Range Transition because of low sample size.

Region	Site	Years	# Plants	# Dates
Intermontane Boreal	Parkinson Site #2 Mulder Yard	2016–2017	20	10
		2016–2017	21	7
		2017–2018	20	4
		2018–2019	20	9
		2019–2020	20	8
	UAF Satellite Dish	2016–2017	20	5
		2017–2018	20	8
		2018–2019	14	8
		2019–2020	26	17
		2017–2018	22	9
	Randy Smith Middle School	2018–2019	20	9
		2017–2018	21	7
		2017–2018	12	13
	Smith Lake Tanana MS Two Rivers Elementary	2017–2018	20	4
		2018–2019	18	9
		2019–2020	13	5
	North Pole MS Weller Elementary	2018–2019	17	16
		2018–2019	9	7
		2019–2020	8	10
	Total	19	341	165
Alaska Range Transition	Birch Hill	2017–2018	20	10
		2018–2019	31	17
		2019–2020	20	7
	East High Environmental Club Mat–Su Career & Technical High	2017–2018	16	11
		2017–2018	20	9
		2018–2019	19	15
		2019–2020	24	10
	Anchorage Botanical Garden Palmer Girl Scout Troop 849	2018–2019	20	13
		2018–2019	19	10
		2019–2020	19	3
	Nanwalek School*	2018–2019	20	2
	Total	11	228	107
Species total	15 sites	30	569	272

APPENDIX 1 TABLE C. *VACCINIUM VITIS-IDAEA*. An asterisk (*) indicates a site was counted as Bering Taiga instead of Bering Tundra due to low sample size. Two asterisks (**) indicates a site was not used in calculations of absolute loss rates because data were not recorded on a per-plant basis.

Region	Site	Years	# Plants in each year	# Dates in each year
Bering Taiga/ Tundra	Bethel Regional High School	2017–2018	21	8
		2018–2019	22	3
		2019–2020	24	6
	Scammon Bay	2018–2019	22	6
	Pilot Point	2019–2020	27	10
	Innoko School	2018–2019	14	11
	Nome Anvil City Science*	2019–2020	58	6
	Total	7	188	50
Intermontane Boreal	Parkinson Yard	2016–2017	20	12
		2017–2018	19	6
	Murie Trail	2017–2018	21	5
		2018–2019	6	3
	Smith Lake	2016–2017	20	5
		2017–2018	20	4
		2018–2019	21	5
	Eagle Community School	2017–2018	25	5
		2018–2019	26	12
	Takotna Community School**	2018–2019	8	6
Coastal Rainforest	Total	10	338	63
	Sitka Raptor Center	2017–2018	40	22
		2018–2019	38	24
		2019–2020	22	14
	Total	3	100	60
Species total	11 sites	20	626	173

APPENDIX 1 TABLE D. *EMPETRUM NIGRUM*. An asterisk (*) indicates a site located in transition zone and counted as Coastal Rainforest instead of as Alaska Range Transitional because of low sample size.

Region	Site	Years	# Plants	# Dates
Bering Tundra	Kamenista	2018–2019	23	4
	Shishmaref School	2017–2018	12	4
		2018–2019		
	Total	3	35	8
Bering Taiga	Innoko River School	2018–2019	8	7
	Scammon Bay School	2018–2019	24	6
	Total	2	32	13
Intermontane Boreal	Parkinson Yard	2016–2017	20	12
	Smith Lake	2016–2017	19	5
		2019–2020	11	4
	Total	3	50	21
Coastal Rainforest	Wynn Nature Center*	2017–2018	23	4
		2018–2019	23	11
	Sitka Raptor Center	2017–2018	31	20
		2018–2019	17	26
		2019–2020	20	15
	Total	5	114	76
Aleutian meadows	Unalaska City High School	2018–2019	19	3
	Unalaska Eagles View	2018–2019	22	9
	Total	2	41	12
Species total	10 sites	15	272	130

APPENDIX 2

PRINCIPAL COMPONENTS ANALYSES (PCA) OF CLIMATE VARIABLES

Climate data were obtained from the SNAP (Scenarios Planning for Alaska + Arctic Planning) at <http://ckan.snap.uaf.edu/dataset/community-charts-temperature-and-precipitation>. We used historical CRU 1961–1990 baseline climatology data for each community with the exception of Twin Rivers (since it was not available, we used nearby Fairbanks instead). All variables were centered and scaled.

The following variables were included in the PCA:

1. Mean daily temperature in January (°C)
2. Mean daily temperature in April (°C)
3. Mean daily temperature in July (°C)
4. Mean daily temperature in October (°C)
5. Number of months with precipitation as snow only (maximum temperature < 0°C)
6. Number of months with precipitation as rain only (minimum temperature > 0°C)
7. Number of months with precipitation as a mix of rain and snow (remaining months)
8. Total annual snow (mm of rainwater equivalent)
9. Total rain (mm)

APPENDIX 2 TABLE A. CHARACTERISTICS AND IMPORTANCE OF PRINCIPAL COMPONENTS.

	PC1	PC2	PC3
Standard deviation	2.3077	1.4084	0.9795
Proportion of variance	0.5917	0.2204	0.1066
Cumulative Proportion	0.5917	0.8121	0.9187

APPENDIX 2 TABLE B. LOADINGS (EIGENVECTORS) FOR THE FIRST TWO COMPONENTS (ABSOLUTE VALUES ≥0.40).

PC1	PC2
Months of snow (0.417)	July mean temp. (−0.535)
October mean temp. (−0.417)	April mean temp. (−0.487)
January mean temp. (−0.404)	Months of rain (−0.479)
Months of mixed precip. (−0.400)	

CBS President's Report for Volume 68

Dear Colleagues,

What an honor and joy it has been to serve as President of the California Botanical Society for the past year! Thank you to all of you for making this such a great year.

First, we have made it through over 20 months of pandemic, and some elements of life are returning to normal. We hope you are all able to get back to the field, lab, herbarium, or wherever you most love to be! Let's keep our fingers crossed things keep heading in the right direction.

Because of the pandemic, the Graduate Student Symposium was online last year, and it was a brilliant success. It was organized by a creative and hard-working team of graduate students from San Diego State University and University of California Los Angeles: Alex Adame, Ioana Angel, Ryan Buck, Samantha Mihalic, Carlos Portillo, Niveditha Ramadoss, and Nelly Rodriguez with support from faculty coordinators Dr. Lluvia Flores-Rentería (SDSU) and Dr. Felipe Zapata (UCLA). The symposium took place over two days at the end of April, with 105 registered attendees, 23 full talks, nine lightning talks, ten posters, and a fascinating keynote presentation on an integrative approach to understanding the evolutionary ecology of California Jewelflowers by Dr. Ivalú Cacho (Universidad Nacional Autónoma de México). Information about award winners and their presentations can be found at <https://calbotsoc.org/past-grad-student-meetings/>. I am excited to announce that this year we will once again hold the Banquet in person in the spring of 2022 – stay tuned for details and registration!

This past year we also started the monthly California Botanical Society's Botany Speaker Series, featuring early career botanists whose research is centered on the flora and environment of western North America. These talks are held on the third Thursday of each month, at 7:00 p.m. Pacific Time and have covered a diversity of topics from restoration of coastal grasslands to moss survival in the desert to a vascular flora of the southern Inyo Mountains to the importance of biological soil crusts. The equally diverse speakers have included graduate students, faculty, and government scientists. The complete list of the ten talks to date, along with recordings, can be found at <https://calbotsoc.org/previous-speakers/>. Please join us for the next talk – all are welcome. Information is at <https://calbotsoc.org/events/>.

This year, as always, we had exceptional applications for the Paul Silva Student Research Grants. We awarded four, to three graduate students and one

undergraduate student. Congratulations to these deserving students and their mentors:

- Emma Fryer, Cal Poly: Community Assembly of Vertic Clay Endemic Annuals of the San Joaquin Desert
- Kristy Snyder, Eastern Washington University, Analysis of the Role of Annual Seeds in Palouse Prairie Restoration
- Riley Scaff, Pitzer (undergrad): A Plant Restoration Study on a Mojave Desert Burn Site
- Annie Taylor, Boise State: Resolving taxonomic uncertainty and clarifying species boundaries in the *Cymopterus terebinthinus* (Hook.) Torr. & A.Gray species complex.

I am excited to welcome several new Council Members (<https://calbotsoc.org/about/>), and for the first time since I started as President, we have a nearly full Council. I'd like to welcome and extend my most enthusiastic thanks to Mitchell Coleman as Membership Chair, Brianne Palmer as Outreach Coordinator, Susan Fawcett as Member-at-Large, Arnold Clifford as Member-at-Large, and returning members Nishi Rajakaruna as Member-at-Large, Brett Hall as Banquet Coordinator, and Ryan Buck as Student Representative. We all also owe thanks to returning Council Members Muriel Poston and Rachael Olliff Yang for assuming the mantles of Corresponding Secretary and Communications Specialist/*Nemophila* Editor, respectively. And it would be a grave oversight if I did not thank our returning and hard-working Council Members Susan Mazer (Past President), Josh Der (First Vice President), Nancy Morin (Recording Secretary), Treasurer (David Margolies), and *Madroño* Editor Justen Whittall. Finally, I must thank Lynn Yamashita, the Society's Administrator, who keeps things running on the right tracks. The Council Members, new and returning, and Lynn, have each contributed to making this past year productive, interesting, full of new knowledge, and fun.

I look forward to seeing you all in person at our spring Banquet! Until then, I hope to see you all at our monthly speaker series. Best wishes for a happy, peaceful, and healthy 2022.



Amy Litt
Current President (2020-2024)
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CBS Editor's Report for Volume 68

I am pleased to report the publication of *Madroño* volume 68 by the California Botanical Society for 2021.

In my fourth year editing *Madroño*, I have attempted to keep *Madroño* at the leading edge of peer reviewed, scientific publications in natural history. Highlights from this past year include publishing 28 full-length Contents articles (our second year in a row of geometric growth), eight Noteworthy Collections (up from five last year), two Book Reviews, and three manuscripts describing New Species. Volume 68 was particularly unique in that it included two Special Issues: "Contributions Toward a Bryoflora of California (and the West), Part V" and "Phenological Patterns in the Flora of Western North America."

In Part V of the "Bryoflora" series, Guest Editor extraordinaire Jim Shevock recruited 12 authors from a range of academic and professional backgrounds, motivated, encouraged, cajoled, bribed, and succeeded in securing six Bryofloras from California, the Pacific Northwest and Wyoming. His reach is so broad, we had to retitle the Bryoflora Special Issue by adding "(and the West)". His leadership, never-ending encouragement, timely reminders, and dedication to perfection shepherded these six manuscripts from mere spores to the mature gametophytes they became in *Madroño* 68(3). At ~200 pages including numerous color plates, extensive checklists, and a thoughtful introduction by Jim, I was sure that this was going to be one of the largest *Madroño* issues I had ever edited. I was wrong.

Phenology is hot! Drs. Rachael Olliff Yang (UC Berkeley) and Jen Yost (Cal Poly) offered to Guest Edit a Special Issue on phenological studies of western plants. The final issue of 2021 consists of 14 Contents (more than we had in all four issues combined last year) and is expected to weigh in at over 200 pages. In this issue, Rachael and Jenn have elevated *Madroño* to a new level of academic rigor by recruiting cutting-edge studies from both established and budding botanists investigating phenology of western North American plants. The results are startling...wherever (and however) you look, most western plants bare the phenological footprints of climate change past and present. We anticipate these studies to be of interest to botanists, ecologists, evolutionary biologists, and climate scientists near and far.

It takes a village to publish *Madroño*. We are grateful for the efforts of 63 distinct reviewers of manuscripts in 2021 (twice as many as last year). We have been actively recruiting reviewers from

increasingly diverse backgrounds to elevate the rigor of *Madroño*. We are increasingly relying on the expertise of botanists from several governmental agencies and private consulting firms in the review process. Along these lines, we have also sought the fresh perspectives students (often in collaboration with their advisors) for reviews. I am especially grateful to reviewers who take the time to truly understand the manuscripts and provide honest, yet constructive feedback to improve the work allowing *Madroño* to maintain its scientific rigor and preeminence among the journals publishing western natural history science. If you are reading this and wondering why you aren't on the list of 2021 reviewers, it's likely because I am not yet aware of your expertise and interest. Contact me with your specialties and I'll get a manuscript to your inbox shortly (madronoeditor@gmail.com) – I look forward to hearing from you.

The efforts of numerous individuals are critical to the successful publication of *Madroño* including a team of Associate Editors and a pair of amazing administrative assistants. Our team of Associate Editors includes Book Editor Matt Ritter, Noteworthy Collections Editor David J. Keil, Copy Editor Alisa Hove, and our Index/TOC Editor Steve Timbrook. Administrative support is provided by Lynn Yamashita and Rachael Olliff-Yang.

The average time from submission to publication for *Madroño* has slowed to ~12 months with some delays due to COVID and the preparation of the two, voluminous Special Issues. We believe that *Madroño* is still the best outlet for western botanists to publish their discoveries in a timely fashion, while reaching a dedicated and passionate audience. I am looking forward to halving this time-to-publication in 2022. We are always on the lookout for future Special Issue topics and Guest Editors. We are currently accepting proposals for Special Issues that highlight any aspect of western botany at any scale of biological organization (from molecules to ecosystems). If you are interested in proposing a Special Issue or would like to discuss your ideas for a Special Issue, please contact me (madronoeditor@gmail.com).

I find great pleasure in helping cultivate manuscripts from the submission process to the printed copy that arrives in our mailboxes and email inboxes. Being on the forefront of discoveries in western botany and working with such astute and passionate authors and reviewers is a pleasure. Botany is alive and well in western North America!

The evidence is in *Madroño*. Please continue to submit your work to the journal, recruit new authors, and share links to the papers often (rather than posting or emailing the PDFs which costs the Society). Like the cool, smooth, peeling bark of *Arbutus menziesii* Pursh, I am shedding our accomplishments of 2021 and look forward to

seeing what botanical discoveries lie in 2022.



Justen Whittall
October 2021

REVIEWERS OF MADROÑO MANUSCRIPTS 2021

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Jessica Wright	Theresa Crimmins
Jim Cohen	Tim Thibault
John A. Christy	William Russel Buck
John Brinda	

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